# The Role of the Deep Roots of Perennial Cereal Kernza in a Drying Climate

a Masterthesis

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# Abstract

Agricultural lands under annual crop production are prone to degradation and as the climate becomes increasingly variable researchers and farmers alike are looking at resilient crops such as perennial grains to produce food regeneratively. Perennial grain crops support a myriad of ecosystem services, such as reducing nitrate leaching, erosion control and increasing carbon storage. With their deep roots, perennial grain crops like Kernza (Intermediate wheatgrass) could furthermore avoid surface stresses such as droughts. This has however not been investigated before. Therefore we set out to determine the depth of root water uptake (RWU) of this crop and compared the contribution of deep roots before and after anthesis and between a year of adequate water supply (2019) and a year of drought (2018). Natural abundances of $^{2}H$ and $^{18}O$ were determined, but were unable to be used properly due to mistakes during sampling. A tracer application showed limited uptake from 2m depth. Furthermore, soil water content measurements were used to inverse model the soil hydraulic parameters under the Kernza crop in Hydrus 1D. Modelling RWU showed that the deep roots (>1m) were responsible for almost 50% of the RWU between anthesis and harvest in 2018, whereas they only contributed between 10% and 15% throughout 2019 and most of 2018 outside of the indicated period. Kernza may thus be an important addition to a farmers toolbox in areas with periodic droughts, but only if grain yields are increased to be competitive with annual cereals or when used as a multifunctional crop for grain, forage and other ecosystem services.

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# Introduction

Annual grain agriculture covers more than 70% of the arable land and caloric intake in our diets worldwide (Glover et al., 2010). Due to increasing global food demands, croplands have become scarce and agriculture has cleared its way into natural ecosystems and forced itself onto marginal lands. More than half of the world population now relies on marginal lands that are particularly prone to degeneration and erosion under the production of annuals grains (Eswaran et al., 1999; Glover et al., 2010). The world population is projected to increase by 50% to 11.2 billion before the end of the century (United Nations, 2017). Because of this and concurrent changes in diets, the global demand for grains is expected to more than double before 2050 (Tilman et al., 2002). Studying deep roots of perennial grain crops may provide a new perspective and a new set of options to meet this growing demand in the future.

Kernza (intermediate wheatgrass) is the first perennial grain crop to be commercially released by The Land Institute. Importantly, yields of this perennial grain crop are still low, typically in the range of 500 to 1000kg $ha^{-1}$ (eg. Culman et al., 2013; Dick et al., 2018). The highest reported grain yield is 1662kg $ha^{-1}$ which equaled 33% of wheat yield in the same study (Culman et al., 2013). Grain yields on average increase from the first year to a peak yield in the second year, but then quickly drops again after the second or third year (Wagoner & Schaeffer, 1990), which is the main reason large-scale support and deployment by farmers is still lacking (Adebiyi et al., 2015). Other reasons Kernza has not been grown by more than a couple of hand full of famers is the lack of knowledge on optimal farming practices. Nevertheless, a farmer's toolbox which includes perennial grain crops could be useful in order to ensure food security and agro-ecosystem functioning under less ideal growing conditions and lower inputs.

Perennial crops may be beneficial due to their crop characteristics and corresponding management procedures. The life cycle of a perennial crop differs from that of an annual crop in several ways. A perennial crop provides a permanent ground cover and living root system. It has an extended growing season compared to annuals which allows it to capture more resources, amongst which is light. Investment in seed production is kept low in the first year after establishment so that the perennating structures can be constructed. The "excess" photosynthesis a Kernza crop performs during compared to an annual cereal is used in subsequent year to recharge these storage organs. (Cox et al., 2010; DeHaan et al., 2005). Critics attribute the low yield of Kernza to a trade-off between seed production and perennation. However, Kernza breeders say this trade-off only exists in the first year when the perennating structures have to be constructed and assign low yield to a lack of domestication traits such as a reduced investment in interspecific competition and an increased harvest index due to the short time since perennial grain crops have started to be domesticated.

Perennial root systems are also capable of expanding year upon year. This results in a number of beneficial ecosystem services provided by perennial grain crops which are not produced by annual grain crops (Cox et al., 2006; Pimentel et al., 2012). One of these benefits may be that crops with deeper roots could be able to reach deeper water stores and capture more precipitation and nutrients than shallow rooting crops. Similarly perennial crops may even be able to capture more precipitation and nutrients than annual crops with similar rooting depths due to their longer growing season and perennial root presence.

At maturity, significant amounts of residual water can still subside in the subsoil under many crops experiencing even severe drought conditions (Passioura, 1983). Deep roots can extend and access deeper reserves of water, thereby improving drought tolerance. As the topsoil becomes water depleted over the growing season, deep roots that have access to moist subsoil become relatively more important (Manschadi et al., 2013). The water uptake during grain filling can affect the yield of a crop to a great extent, small amounts of water extracted after the onset of flowering can lead to relatively large differences in grain yield. So much so that the marginal water use efficiency of water taken up post-anthesis is about two times as high as calculated over the whole growing season for wheat (Manschadi et al., 2013). Every mm of water that is taken up after the onset of flowering can result in up to 55kg of extra yield per hectare in wheat (Manschadi et al., 2006). Indeed, drought resistant varieties of crops often have deeper roots than their drought susceptible counterparts (Christopher et al., 2008; Manschadi et al., 2006; Manschadi et al., 2013). Furthermore, traits optimizing water uptake also increase the capture of highly mobile and dissolved nutrients like nitrate (Dunbabin et al., 2003).

Simultaneously with increasing land-use change pressure, climate change will cause increasing variable climatic conditions and increased frequency of extreme weather events. Deep roots, defined by Maeght et al. (2013) as roots growing at depths below 1m, may however reduce the influence of surface stresses such as periodic droughts on crop growth. Crops may avoid these stresses by furrowing their roots into the deep soil where surface fluctuations abate. Besides, water and nutrient availability are limiting factors in almost all agro-ecosystems, thus increasing the capability of roots to acquire and use these resources efficiently may be the key to solving many agricultural sustainability problems at once (Lynch, 1995).

So far however, little emphasis has been placed on exploiting the root phenome to improve crops (Lynch, 2007). Consequently, little attention has been paid to deep roots in scientific research. Most of the existing crop root research has focused on the roots in the upper 1m of the soil, neglecting the influence of roots below this horizon (Pierret et al., 2016). Although the popularity of these subjects has been increasing, with a search in the Web of Science™ database Pierret et al. (2016) found only 767 and 276 references for the search terms “deep root and plant” and “deep rooting” respectively,

compared to more than 48000 references to “plant root” and 8200 references to “fine root”. It is very probable that only a fraction of the deep root references concerns crops.

Additionally Rothfuss and Javaux (2017) found that the depth of root water uptake (RWU) is rarely directly determined by using of stable isotopes, with only 159 studies corresponding to the search terms “root water uptake” or “water source” and root or “water uptake” in combination with “isotop*” in the last 32 years of which only 15% were studying RWU of agricultural crops and 32% used multi-source mixing models to determine source contribution to RWU.

In conclusion, on the doorstep of this thesis a number of fringe research areas meet that still lack the investigative effort up until this point. Considering the aforementioned benefits of perennialism and deep roots and the importance of deep roots in water acquisition, studying perennial grains crops, deep root profiles and RWU become of utmost importance.

## Research question

The contribution of deep roots to plant water uptake remains largely unknown. This is specifically true for Kernza, a new perennial crop whose ecology and physiology have yet to be thoroughly uncovered. Therefore with this thesis I will address the following research question including the subsequent sub-questions:

**To what extent do deep roots contribute to the total root water uptake of the perennial cereal Kernza?**

- What is the maximum rooting depth and root length density profile of Kernza on our location?
- What is the relative contribution of deep roots to the total root water uptake before and after anthesis?
- What is the relative contribution of deep roots to the total root water uptake during a dry growing season versus a relatively wet growing season?

## Hypothesis

Based on volumetric water content measurements using TDR sensors from the past year where changes in water content are slightly visible at 1.5m depth but not at 2.5m depth, the maximum rooting depth is expected to lie between these depths, although Kernza is known to be able to root deeper than 3m (Oliveira et al., 2018). The root length density is expected to follow an exponential decrease with depth. Root water uptake is expected to be strongly correlated to RLD when water is available along the full soil profile. The deep roots are hypothesized to contribute relatively more to root water uptake after anthesis as the topsoil is expected to be relatively dry during this period (Manschadi et al., 2013). In the same vein, during the dry summer of 2018 deep roots are anticipated to be relatively more important than during the relatively wet summer of 2019.

# Materials and methods

## Site description, soil characteristics and crop management

The research for this thesis has been conducted at the DeepRootLab of the University of Copenhagen (Latitude 55.66815°N, Longitude 12.30848°E), in Taastrup, Denmark, at an elevation of 26 m above sea level. The soil had a sandy loam texture in the top horizon and changes to a loam deeper in the profile (ASDA classification; table 1). Climatic conditions were recorded at an on-site weather station. The mean annual precipitation is 750mm and the mean annual temperature were 9,6$^{o}$C. Under the experimental field a network of drainage pipes, wells and sensors was constructed in 2016. Three replicated plots of 100m2 (10 by 10m) Kernza from the 5$^{th}$ selection cycle (DeHaan et al., 2018) were sown in April 2016 at 20kg ha$^{-1}$ with a row spacing of 25cm. The stands were three years of age by the time this research was conducted. Plots were fertilized with 100kg N ha$^{-1}$ in July 2016 and with 177 kg ha$^{-1}$ (YaraMila 21-7-3) in spring 2017. In May 2018 an equivalent of 171.5kg ha$^{-1}$ NPK fertilizer (Danish Agro 21-3-16) was applied to the plots.

*Table 1 Soil properties*

| Soil depth (cm) | P (mg/kg) | K (mg/kg) | Mg (mg/kg) | SOM (%) | Clay (%) | Silt (%) | Fine Sand (%) | Coarse Sand (%) | Total Sand (%) | Total N (%) | pH | Bulk Density (g/dm3) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-25 | 5,7 | 12 | 5,2 | 1,3 | 13 | 14,4 | 42,9 | 29,5 | 72,4 | 0,08 | 7,8 | - |
| 25-75 | 1,3 | 8,4 | 7,8 | 0,5 | 17,1 | 14,5 | 41,8 | 26,1 | 67,9 | 0,03 | 7,9 | 1,74 |
| 75-150 | 0,5 | 7,3 | 7,1 | 0,2 | 19,1 | 19,5 | 34,6 | 26,6 | 61,2 | <0.03 | 8,2 | 1,75 |
| 150-300 | <0.4 | 6,3 | 8,1 | 0,1 | 18,7 | 19 | 35,3 | 26,9 | 62,2 | <0.03 | 8,1 | 1,83 |
| 300-450 | 0,4 | 8,4 | 11 | 0,3 | 20,7 | 22,6 | 32,3 | 24,1 | 56,4 | <0.03 | 7,8 | 1,83 |

## Root measurements

### Soil coring

To assess the root length density (RLD) distribution, two soil coring campaigns were conducted, one early June and again one early July 2019. During these campaigns a single vertical soil core was drilled in each plot to a depth of 2 meters using an Edelman hand-auger (Eijkelkamp, ø7cm). Soil samples were taken for every 25cm. A fraction (300-400g) of each sample was taken apart so that it could be used for both water extraction, subsequent $\delta^2H$ determination and for soil moisture content measurement. The larger part of the soil sample was taken for root washing followed by RLD determination. Both parts were stored in zip-lock bags, weighed and frozen. Thus, in total six soil cores were taken adding up to a total of 48 soil samples corresponding to 25cm depth intervals.

### Root washing

After the soil cores had been taken, roots were washed in the next step to uncovering the RLD. Over the course of two weeks in August, batches of soil samples were let to defrost in a cold room at 6$^{o}$C. Defrosted samples were soaked in buckets of water and if needed, stirred to break up aggregates and

clumps until a uniform suspension of soil particles was achieved and roots were no longer retained in the soil matrix. At this point the soil-water suspension was continually stirred and poured through a sieve (0.5mm mesh). Soil particles were washed away with water but root pieces and other debris were caught in the sieve. When no roots were detected in the suspension anymore, the sieve was emptied in a second bucket. Roots were then separated from other non-root debris using a smaller secondary sieve and tweezers and were stored in 70% ethanol in a fridge at 4$^{o}$C.

### Root scanning

The obtain the RLD, the root samples were than scanned with a scanner (Epson Perfection V700 Photo) and analyzed using the WinRHIZO software package (Regent Instruments Inc., 2015). Roots were placed uniformly, away from the edge and in adequate amounts at a time in a square plastic container. A scanning image (800dpi, 8x10inch) was then made and this image loaded in WinRHIZO. In WinRHIZO the image was analyzed using automatic background distinction and a debris filter for material with a length/width ratio smaller than 4. This way, small scratches on the plastic container and non-root debris that were not sieved out during root washing, were filtered out during analysis. Other settings were kept at default. WinRHIZO compiled a text file in which the total root length and root length density were of interest to this study.

## Isotope experiment

Two methods were used to determine root water uptake (RWU) of Kernza in the field, 1) using natural $^{2}$H and $^{18}$O abundance and 2) an enriched $^{2}$H tracer (figure 1).

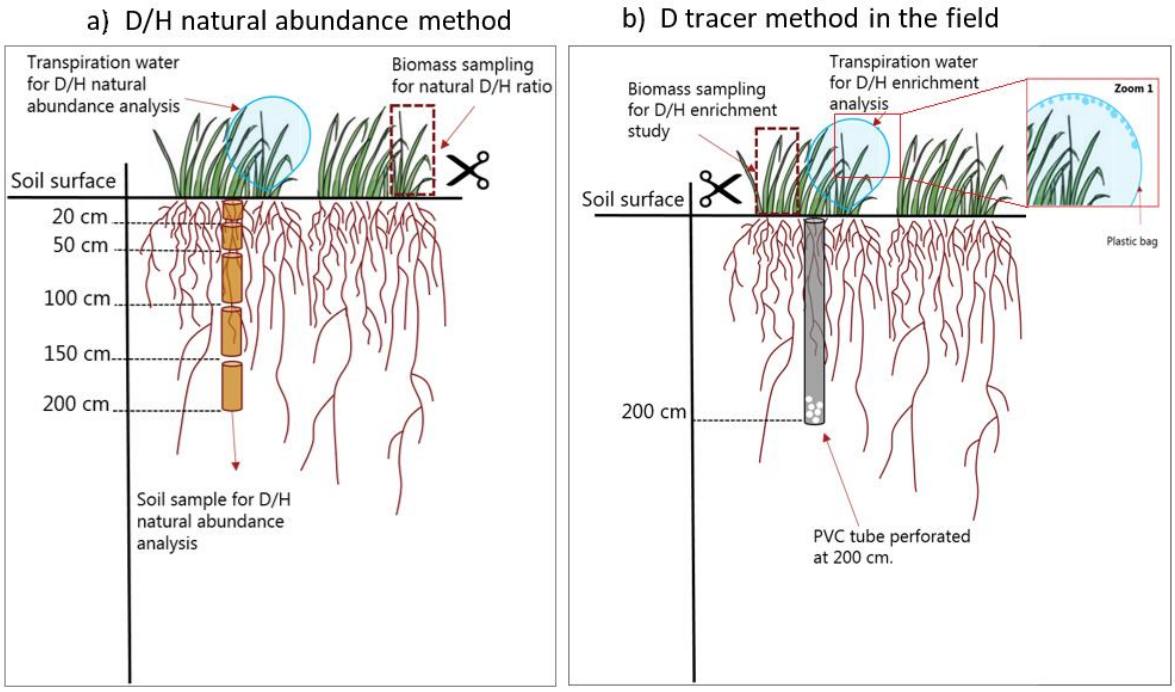


*Figure 1 Scheme of the stable isotope methods that were used to estimate the crop water uptake depth. a) D/H natural abundance in the soil b) D field tracer injection at 2.0 m depth. The figure displays the types of samples that will be collected.*

### Natural abundance of $^{2}H$ and $^{18}O$ sampling

Of each 25cm soil sample from the soil coring, between approximately 300-400g of soil was kept separate and frozen for natural abundance isotope analysis. These soil samples were used to construct natural abundance soil profiles. The groundwater was sampled a priori and rain water samples were taken after every rain event from a bucket that was placed between the experimental plots. Furthermore, during every sampling day of the isotope experiment, control biomass and transpiration water samples were taken. These control samples served a dual purpose. They functioned as control samples to determine background $^{2}H$ and $^{18}O$ to compare the tracer samples with, but they were also to be used in a mixing model to determine the depth of RWU.

### $^{2}H$ tracer application and sampling

After the soil coring was completed, opaque PVC pipes (2.1m in length, ø6cm) were inserted into each hole. These tubes were open ended with an additional eight holes (ø10mm) drilled in the bottom of the tube to ensure that the tracer was able to move into the surrounding soil (Fig 1). The tubes were hammered into place after which it was made sure they reached the two-meter-depth mark using a measuring stick. The tops of the tubes was then capped with PVC caps and the space around the PVC tube was sealed with bentonite, an expanding clay that prevents selective water infiltration around the tube.

After the PVC pipes were installed, a deuterium tracer ($\delta^{2}H_{june}$=300000‰, $\delta^{2}H_{july}$=600000‰ ) was carefully applied at the bottom of the tubes, using a smaller PVC pipe and a funnel. In the morning of the subsequent days transpiration water was collected from the plants growing immediately around the PVC pipe as well as from a random control location in the plot. This was done by putting a plastic bag (80L, Logicon Nordic A/S, EM-630022) over a handful of tillers and tying the bag at the bottom with a string in such a way to create a small gulley so that water can collect at the bottom without leaking out. In the afternoon of the same day the plastic bags were carefully removed without spilling and the collected water was transferred to a plastic container using a separate disposable plastic pipette for each sample and stored in a fridge. At the same time biomass samples (3-4 tillers per sample, without inflorescence) were

taken of plants growing directly around the PVC tube and also from a random control location in the plot. These samples were placed in test tubes (Pyrex, 1622/22M) and were then capped (Kartell Labware, ø22mm) to prevent evaporation and after all samples were taken, frozen to stop transpiration and prevent decomposition. This entire process, from soil coring to freezing biomass samples was repeated in July (for planning see Table 2).

From each vial/container of transpiration water a sample was taken using a syringe (B Braun Omnifix-F, 1mL) and put through a filter (Q-Max syringe filters, ø 13 mm) into a autosampler

*Table 2 Sampling schedule, numbers represent amount of samples taken, numbers in red represent samples which were later actually analyzed.*

| Timing | Day | T-4 | T0 * | T+1 | T+3 | T+4 | T+8 | T+24 | T-3 | T0 * | T+2 | T+4 | T+7 | T+16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Date | 13-6-2019 | 17-6-2019 | 18-6-2019 | 20-6-2019 | 21-6-2019 | 24-6-2019 | 11-7-2019 | 18-7-2019 | 22-7-2019 | 24-7-2019 | 26-7-2019 | 29-6-2019 | 7-8-2019 |
| Transpiration water (2ml) | PVC injection | | | 3 | 3 | 3 | 3 | 3 | 3 | | 3 | 3 | 3 | 3 |
| | Natural abundance /Control | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Plant biomass (approx: 20g) | PVC injection | | | 3 | 3 | 3 | 3 | 3 | 3 | | 3 | 3 | 3 | 3 |
| | Natural abundance /Control | | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Soil (Natural abundance) 300g | Soil | 24 | | | | | | | 24 | | | | | |
| Note: *Injection at the end of the day | | | | | | | | | | | | | | |

vial (Fisherbrand, 2mL). This was done to filter out any organic particles thereby prepare the samples for mass spectrometry. All samples, soil, transpiration and biomass were packed in styrofoam boxes topped off with sufficient dry ice and send with priority mail to the SilvaTech lab in Champenoux, France.

## Sample processing and analysis

The soil, biomass and water samples were processed and analyzed during the course of a two-week residency in the SilvaTech lab at the French National Institute of Agricultural Research (INRA), location Champenoux, in September. Of all samples, a selection was made due to limitation in finances and time (table 2, numbers in red).

Transpiration water samples, which were in no need of further processing could be analyzed immediately. To avoid any memory effect in the mass spectrometers, analysis started with ground, tap and rainwater and water from control samples, then moving to samples from the tracer experiment where enrichment was expected. The water from the soil and biomass samples were extracted using a cryogenic vacuum distillation apparatus.

After the sampling campaigns were done and extraction/analysis was about to commence, we found out we should not have sampled general biomass, which included both stems and leaves, since leaves have a isotopic composition which differs from the source water represented in the stems. Before extraction therefore we separated stems from leaves from the frozen samples in a cold room. For some sample dates (18-6, 18-7 and 24-7) leaves and stems were analyzed separately to check for a mixing effect due to sampling and storing of both organs together in the same test tubes. For the other dates only stems were analyzed. For the data to qualify for use in a mixing model, first it was determined whether the isotopic composition still fell within range of the isotopic composition of the soil profile. Also a dual-isotope plot was constructed to check for the effect of fractionation and mixing of leaf and stem water.

Some samples were double checked using both Picarro and IRMS mass spectrometers to check for accuracy and contamination with dissolved organic matter. No contaminations were found in any of the sample types after which the choice was made to continue processing with Picarro only due to time and cost-effectiveness. Moreover, Picarro outputs both the $\delta^{2}H$ and the delta $\delta^{18}O$ simultaneously, while the IRMS would only be able to return one of these isotope ratios at once, making it both time and cost effective. Delta values were determined in relation to the VSMOW international standard:

Eq. 1 $$\delta = 1000‰ \cdot \frac{R_{sample} - R_{VSMOW}}{R_{VSMOW}}$$

where R stand for the ratio of the heavy over the light isotope.

### Soil water content

Only a small fraction of each soil sample was needed for water extraction and subsequent mass spectrometry. The remaining portion of each sample was weighed, then oven dried at 105$^{o}$C for 48 hours and then weighed again for determining the soil water content of the soil profiles at the time of soil coring in July.

### Data analysis

First the different sample types were plotted in a dual isotope plot. Linear regressions were performed per sample type and the regression coefficients were compared with an ANOVA in SPSS. Mean delta values for each sample type were also compared with an ANOVA. These mean delta values for the stem samples were plotted in the graph for the delta value of the soil profile. No multisource mixing model was applied because fractionation an mixing of water sources occurred post-sampling.

## Root water uptake modelling

The macroscopic Soil-Plant-Atmosphere Continuum model Hydrus 1D (PC-Progress) was used to model one-dimensional soil water flow and root water uptake. In this model the macroscopic Richards equation is solved for a sink-term which represents root water uptake:

Eq. 2 $$\frac{\partial \theta}{\partial t} = \frac{\partial}{\partial z}\left[K(h)\frac{\partial h}{\partial z} - K(h)\right] + S$$

where θ is the volumetric water content (cm$^3$ cm$^{-3}$), h is the soil water pressure head (cm), K represents the soil water hydraulic conductivity [cm day$^{-1}$], *S* is the sink term [cm$^3$ cm$^{-3}$day$^{-1}$], z is the vertical positive position (cm), and t is the time (day).

However a more intricate soil hydraulic model was chosen which performed better, namely the the dual van Genuchten and Mualem model introduced by Durner (1994). This dual-porosity model assumes a non-uniform pore size distribution in the soil with different retention characteristics for the separate compartments, which is a more realistic description of a soil. This dual porosity model looks as follows:

Eq. 3 $$\mathrm{K}(\mathrm{S}_e) = K_s \frac{(w_1 S_{e1})^l \left(w_1\alpha_1\left[1-\left(S_{e1}^{\frac{1}{m1}}\right)^{m1}\right] + \left(w_2\alpha_2\left[1-\left(S_{e2}^{\frac{1}{m2}}\right)^{m2}\right]\right)^2}{(w_1\alpha_1 + w_2\alpha_2)^2}$$

where $K_s$ is the saturated hydraulic conductivity, $S_e$ is the effective water content, $w_i$ are the weighting factors for the sub-curves of two compartments in the dual porosity model, and $a_i$, $n_i$, $m_i$ (=1-1/$n_i$), and $l$ are empirical parameters of the sub-curves.

No hysteresis in the retention curve was allowed for, because no separate wetting and drying

retention curves were measured. Furthermore, water uptake was linearly reduced in cases of water stress using the Feddes water stress parameters (Feddes & Raats, 2004). These parameters represent the pressure head at which root water uptake is optimal as well as when root water uptake is terminated due to anoxia or drought, assuming a linear reduction between these points. The Feddes parameters differ from crop to crop, but are known for only a limited number of crops. They are known for perennial rye grass and for wheat, but not for Intermediate Wheatgrass/Kernza. Since Kernza physiologically resembles a perennial grass more than an annual cereal, the Feddes parameters for grass were selected.

The depth of the soil profile was set at 250cm and was divided into 2 materials based on measured textural properties (table 1). The first material started at the surface and extended to 75cm depth. The second material started at 75cm and stretched until the bottom of the profile at 250cm.

### Atmospheric boundary conditions

To simulate the atmospheric conditions, daily precipitation, potential evapotranspiration and hCritA values were entered. The potential evapotranspiration for Kernza was kept the same as the reference evapotranspiration as no crop coefficient ($K_c$) is available for Kernza nor for Intermediate wheatgrass in general to transform reference into crop specific evapotranspiration.

The critical pressure head (hCritA) at the soil surface at which the actual evaporation is not equal to the potential evaporation anymore, due to the soil surface being too dry to supply water for evaporation and is calculated using the following equation:

Eq. 4 $$hCritA = \frac{RT}{Mg} \ln (H_r)$$

where M is the molecular weight of water (=0,018015 kg $mol^{-1}$), g is the gravitational acceleration (=9.81 m $s^{-2}$) and R is the gas constant (=8.314 J $mol^{-1}$ $K^{-1}$), T is the temperature in Kelvin and $H_r$ the relative humidity.

Furthermore, the groundwater level was used as a variable lower boundary condition and leaf area index (LAI) was selected as input. The Hydrus code uses the LAI to divide the measured evapotranspiration into a separate evaporation and transpiration component. After which the hCritA is used to reduce potential evaporation to actual evaporation and the Feddes water stress parameters are used to reduce potential transpiration to actual transpiration. LAI for Kernza was determined using the growth stages which were recorded according to the mean stage count (MSC) system for describing growth stages of Moore et al. (1991) and the formula of Mitchell et al. (1998) to calculate LAI from MSC:

Eq. 5 $$LAI = -11{,}54 + 18{,}45x - 4{,}198x^2$$

where x is the MSC-value.

MSC was recorded on an approximately bi-weekly basis, linear interpolation was used to obtain MSC values for the dates between measurement days. The LAI is then used in Hydrus 1D to separate the potential evapotranspiration into a separate potential transpiration and potential evaporation using the following equations:

Eq. 6 $$T_p = ET_p(1 - e^{-k*LAI})$$

Eq. 7 $$E_p = ET_p\, e^{-k*LAI}$$

where k is a constant governing the radiation extinction by the canopy.

An extinction coefficient (k) of 0.5 was used. Lastly, a maximum pressure head of 2cm was allowed to build up at the surface before run-off is initiated, because in winter a small pool of water has been seen to form in the area where the plots are located.

### Initial conditions

Initial conditions were specified according to the volumetric water content value given by the TDR sensors at the date of the beginning of the simulation. In case of the forward simulations which started on 1-1-2018 when no TDR was installed yet, the initial condition was assumed to be the saturated water content, as on 1-1-2019 this is also the case. The root length density obtained from the root washing and scanning was kept constant throughout the simulation. Within every 25cm depth interval RLD was assumed to be equal.

### Soil hydraulic parameters and inverse modelling

Retention curves were previously empirically measured from soil from 75cm and 150cm depth. The program RETC (PC-Progress) was used to inverse model the primary soil hydraulic parameters used in the dual-porosity model from the measured retention curves. The produced primary soil hydraulic properties were used as a fixed input in the Hydrus 1D model. Hydrus was then used to inverse model the hydraulic conductivity and the secondary soil hydraulic parameters of the dual porosity soil hydraulic model.

Importantly, the inverse modeling of the soil hydraulic parameters was first done on a different crop, namely alfalfa. Alfalfa was grown next to the Kernza plots and the same data were measured. However, for alfalfa it was possible to construct a crop coefficient ($K_c$) curve and thereby inverse model more accurately than would be possible with the Kernza model, for which no $K_c$-values are known. The obtained soil hydraulic parameters were then used as input in the Kernza model. The choice was made

to inverse model and thus optimize the hydraulic conductivity ($K_s$) and saturated water content for the secondary retention curve ($w_2$) further in the Kernza model to improve fitting. The other remaining soil hydraulic parameters (fitting parameters $l$, $\alpha_2$ and $n_2$) were kept the same as in the alfalfa model. Model performance was superior when these fitting parameters were kept the same and only $K_s$ and $w_2$ were optimized further.

Volumetric water content data from TDR sensors in each plot at 75, 150 and 250cm depth were used to fit the model with and thereby optimize the above mentioned parameters. Since the TDR sensors were installed and activated in the spring of 2018, the inverse modelling was started at 11-5-2018 until 23-9-2019.

### Simulating root water uptake

Finally, after inverse optimization of all soil hydraulic properties, the model was run to acquire the root water uptake for every centimeter of the soil column, starting at 1-1-2018 until 23-9-2019. This time series encompasses the two periods of interest, namely the growing season for each year. A modified version of Hydrus was made which allowed for the creation of 200 observation nodes and the root water uptake was modelled for every node at which roots were present. The obtained data was modified in Excel and graphs were produced using, MatLab and Excel. The depth of root water uptake during the growing periods of 2018 and 2019 were then compared.

## Harvest

Kernza was harvested on the 12$^{th}$ of August. Due to the destruction of a part of each plot due to machinery used in another experiment, it was decided that instead of harvesting a difficult to determine intact area all at once by combine harvester, several samples would be taken. Using a 1/4m2 metal square and a sickle, four 1/4m2 areas were harvested in random locations inside each plot at a height of 5-10cm. The harvested biomass was then put in cotton bags and left to air dry above a warm vent for nine days. On the 21$^{st}$ of August the air dried Kernza was threshed (Wintersteiger LD350 thresher). A 1.5 x 4.5 mm filter was used to separate chaff and other material from grain. To completely dehull the grains, they were put through the thresher 3 times. At this point the grain was sufficiently clean so that additional cleaning was obsolete. The few non-grain particles left in the grain container were picked out by hand, which was possible due to the small amount of harvest.

Straw and grain were then oven-dried for 48 hours at 80$^{o}$C. Thereafter straw and grain were weighed and yields were averaged and extrapolated to represent the yield per hectare. Harvest index and thousand kernel weight were determined subsequently.

# Results

## Soil Coring

The RLD of Kernza followed a near exponential decline with depth. Root length densities were averaged over the three plots and over the soil coring campaign in June and July, as differences were likely to be caused by placement of the soil core (coincidentally in proximity or distance from roots) and not by plot number or date. In the topsoil the RLD reached a mean value of 7.7 $cm/cm^3$ declining to a mean RLD of 0.09 $cm/cm^3$ in the interval from 1.75- 2m. Roots could have been found even deeper would cores have extended 25cm deeper.

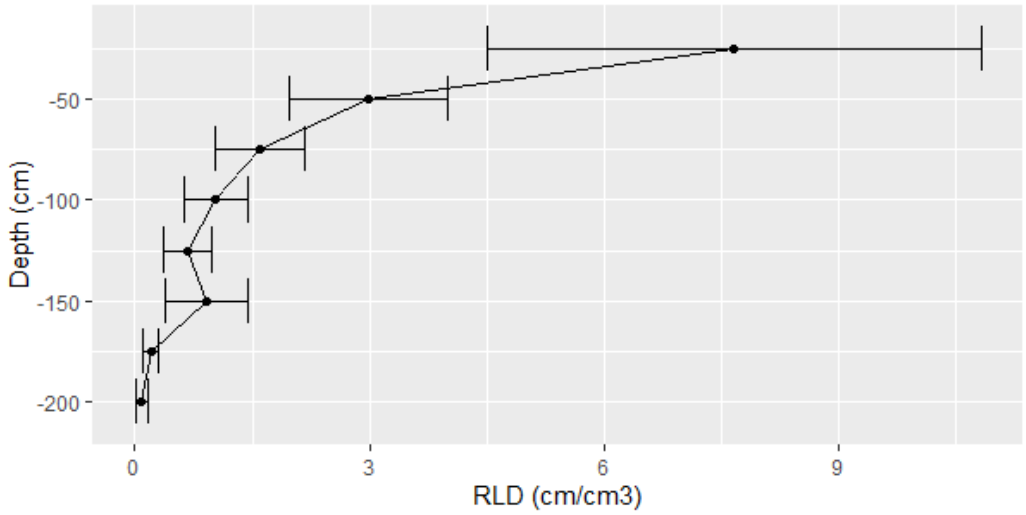


*Figure 2 Mean RLD of Kernza obtained from the soil coring and root washing campaign in the three plots in June and July of 2019. Error bars represent the standard deviation.*

## Isotope experiment

### Natural abundance

The natural abundance of the different sample types was assessed and presented in figure 3. For both stable isotopes leaves were relatively enriched compared to stem samples, although this difference was not found to be significant (table 3). Transpiration water δ-values for both stable isotopes were significantly lower than the corresponding δ-values of the stem samples. Furthermore, in both cases transpiration water samples were, in terms of isotopic enrichment, significantly identical to rainwater samples. Groundwater samples showed the highest relative depletion, although this did not differ significantly from the δ-values of both leaf and stem water samples in the case of $^2H$.

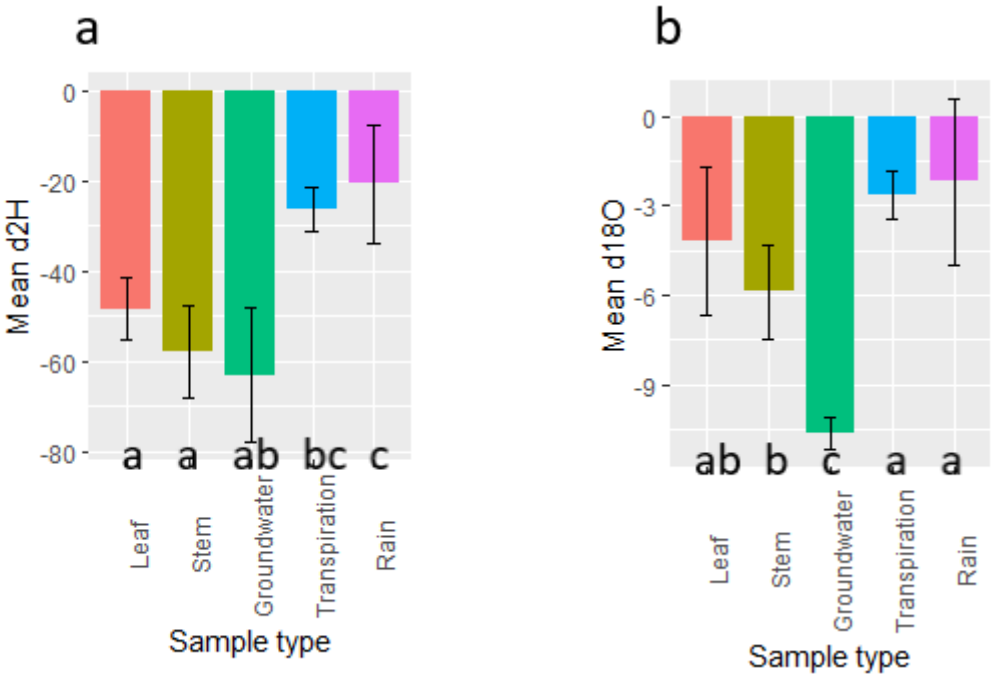


*Figure 3 Mean δ-values of leaf, stem, transpiration rain and groundwater natural abundance samples. Letter indicate whether difference were found to be significant or not.*

*Table 3 P-values of the ANOVA pairwise comparison post hoc test with a Bonferroni correction. Table a) corresponds to figure 3a and displays p-values for mean $\delta^2H$ comparison, table b corresponds to figure 3b and displays p-values for mean $\delta^{18}O$ comparison.*

| **a** | Leaf | Stem | Groundwater | Transpiration | Rain water |
|---|---|---|---|---|---|
| Leaf | - | 0.103 | 1.0 | <0.001 | 0.001 |
| Stem | | - | 1.0 | <0.001 | <0.001 |
| Groundwater | | | - | 0.138 | 0.041 |
| Transpiration | | | | - | 1.0 |
| Rain water | | | | | - |

| **b** | Leaf | Stem | Groundwater | Transpiration | Rain water |
|---|---|---|---|---|---|
| Leaf | - | 1.0 | 0.0033 | 1.0 | 1.0 |
| Stem | | - | <0.001 | <0.001 | 0.038 |
| Groundwater | | | - | <0.001 | <0.001 |
| Transpiration | | | | - | 1.0 |
| Rain water | | | | | - |

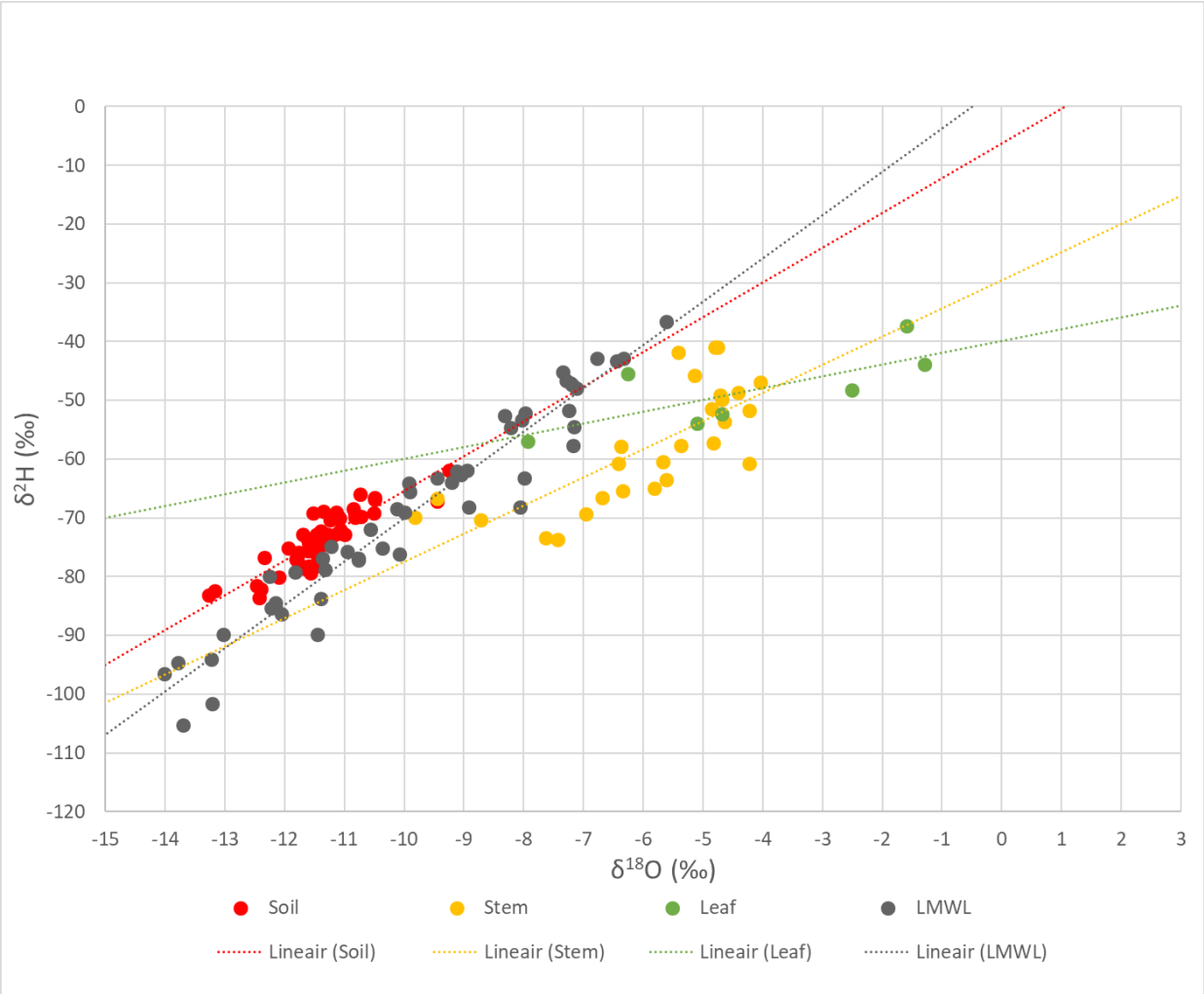


*Figure 4 Dual isotope plot of local meteoric water line (LMWL) and soil, stem and leaf samples. The equation at the top represents the regression line of the LMWL. Water in the soil and stem should in theory be similar to meteoric waters. Leaf should deviate due to fractionation at the evaporative sites along the leaf surface. Slope difference in relation to source water indicate fractionation has occurred.*

A dual isotope plot was used to assess the degree of fractionation which happened post-sampling. The grey line represents the Local Meteoric Water Line (LMWL) which is a regression from measured water isotopic ratios (IAEA, 1974). Leaf samples formed a significantly different regression slope compared to soil and stem (ANOVA, p=0.01 and p=0.03, respectively ). The stem samples showed a small decrease in slope in respect to the soil samples, although this difference was not significant (ANOVA, p=0.27). The mean delta values between soil and stem however did differ significantly, which indicated mixing with fractionated leaf water.

Consequently, the delta values for the natural abundance of $^{2}H$ and $^{18}O$ were plotted against the corresponding delta values of the soil profile from the soil coring campaign in July (fig. 5). In both cases, but more extremely so in the case of $^{18}O$, the mean delta value of the sampled stem water did not fall within range of that of the soil profile, a requirement for the application of a multi-source mixing model. The standard deviation of the delta value of the stem water samples manages to only touch the standard deviation of the delta value of the bottom two compartments of the soil profile from 1.5-2m depth.

The volumetric water content of the soil profile at the time of sampling in July was displayed in figure 7. The top 50 centimeters of the soil are very dry but the moisture level increases with depth.

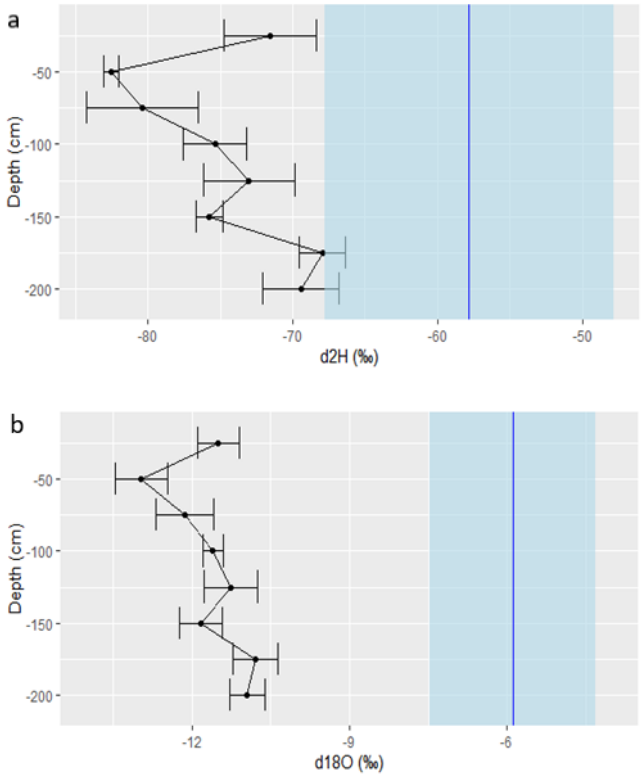


*Figure 5 Soil profile isotopic values and mean stem isotopic value (blue line) for a) $^{2}H$ and b) $^{18}O$ from the natural abundance data of the July sampling campaign. Blue shaded area represents the standard deviation from the stem sample mean.*

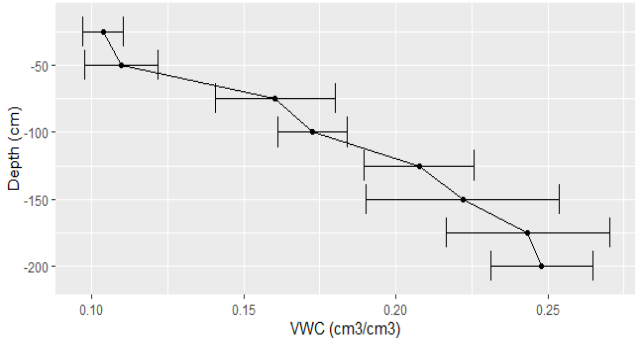


*Figure 6 Mean volumetric water content ($cm^3\ cm^{-3}$) of the soil profile of the three plots per 25cm interval from the July soil coring campaign. Error bars represent the standard deviation.*

## Tracer Experiment

In figure 7 it is shown that enrichments due to tracer uptake only occurred at day 16 after tracer injection in July. This signal was only recorded in the transpiration water sample of this date. On none of the other dates an enriched signal of tracer uptake was found.


*Figure 7 $\delta^2H$ results from the twice performed deuterium tracer experiment. After injection of the tracer at the bottom of the PVC tubes, samples were analyzed for the 4th, 8th and 24th day after injection in June and the 4th, 7th and 16th day after injection in July. The control values represent the mean value of the control samples taken over the experimental period.*

## Modeling root water uptake in Hydrus 1D

### Inverse modeling soil hydraulic parameters

Figure 8 displays the results of the fitting of a retention curve through measured retention data points. The fitting automatically set the residual water content to zero and continued the fitting with this in mind. From the fitted retention curve, RETC then estimated the saturated water content, α and n, the latter two being fitting parameters (table 4). Figure 8a represents the retention curve of the soil at 75cm depth (model performance: Sum of squares (SSQ) = 0.00267, $R^2$ = 0.91) and figure 8b represents the retention curve for soil at 150cm depth (SSQ = 0.00076 $R^2$ = 0.98). The predicted soil hydraulic parameters from table 4a were used to represent the first material (0-75cm depth) in the Hydrus 1D model and the predicted soil hydraulic parameters from table 4b were used to represent the second material (75-250cm depth) in the Hydrus 1D model.

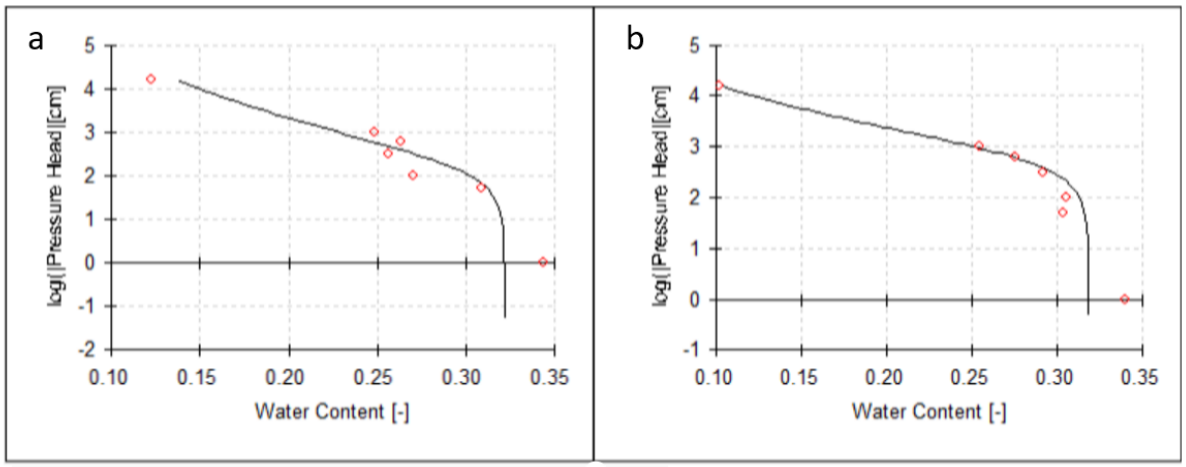


Figure 8 Inverse modelling of soil hydraulic parameters in RETC by fitting retention curve through measured retention data points (in red). Figure a. is the retention curve for the soil at 75cm depth, figure b for 150cm depth.

Table 4 Predicted soil hydraulic parameters. ThetaS is the saturated water content, Alpha (α) and n are fitting parameters.

a

| Variable | Value | S.E.Coeff. | Lower 95% limit | Upper 95% limit |
|---|---|---|---|---|
| ThetaS | 0.3218 | 0.2009 | 0.266 | 0.3775 |
| $\alpha_1$ | 0.0055 | 0.0056 | -0.0101 | 0.021 |
| $n_1$ | 1.1918 | 0.0692 | 0.9996 | 1.384 |

b

| Variable | Value | S.E.Coeff. | Lower 95% | Upper 95% limit |
|---|---|---|---|---|
| ThetaS | 0.3181 | 0.0083 | 0.2951 | 0.3412 |
| $\alpha_1$ | 0.0013 | 0.0004 | 0.0001 | 0.0025 |
| $n_1$ | 1.3751 | 0.0684 | 1.1852 | 1.565 |

In the Hydrus 1D model the soil hydraulic parameters were used as input and were not further optimized. The additional soil hydraulic parameters were inverse modelled in Hydrus 1D by fitting the model with observed TDR data (fig. 9), using a different crop, alfalfa, for which the crop coefficient was known the soil hydraulic parameters were inverse modelled (Root Mean Square Weighted Error, RMSE = 0.0067, $R^2$ = 0.94) . These were than used in the Kernza model. The hydraulic conductivity ($K_s$) and saturated water content of the secondary porosity compartment ($w_2$) were further optimized in the Kernza model (RMSE = 0.8737E-02, $R^2$ = 0.90), the other ones were kept constant.

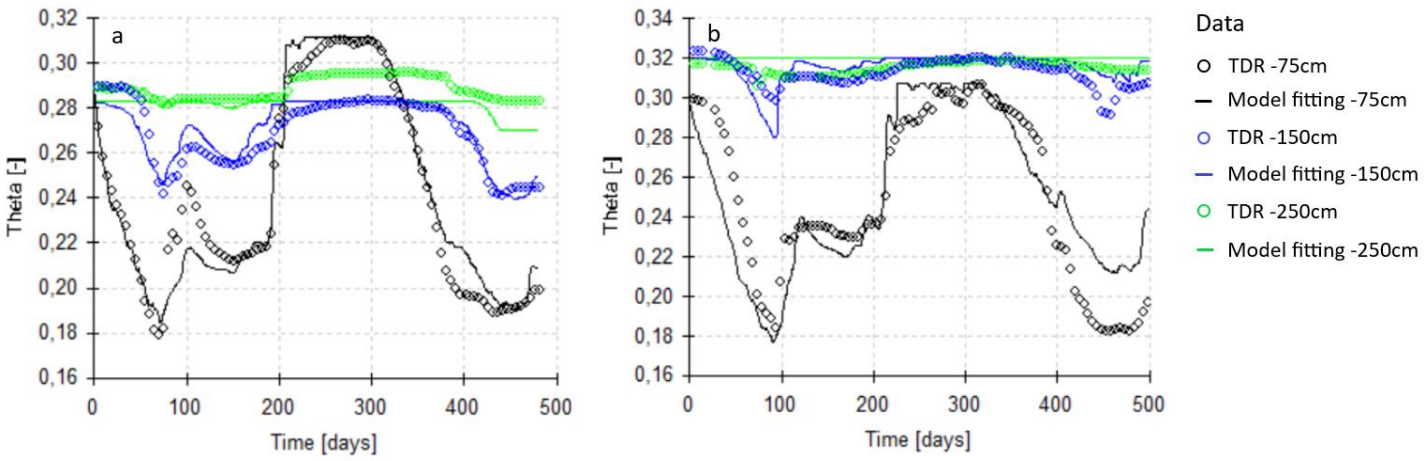


Figure 9 Fitting of Hydrus 1D predicted volumetric water content data with observed TDR volumetric water content data. Figure a. represents the inverse modelling using alfalfa for more accuracy in the parameter prediction. Figure b represents additional fitting using the Kernza model.

Table 5 Predicted soil hydraulic parameters. $K_s$ is the hydraulic conductivity, $l$ is a fitting parameter. $w_2$, $\alpha_2$ and $n_2$ represent soil hydraulic parameters of the secondary soil compartment in the dual porosity model.

| | | | | 95% Confidence limits | |
|---|---|---|---|---|---|
| Material | Variable | Value | S.E.Coeff. | Lower | Upper |
| 1 | $K_s$ | 2.4292 | 0.067 | 2.2977 | 2.5607 |
| 1 | $l$ | 2.9152 | 0.1809 | 2.5604 | 3.27 |
| 1 | $w_2$ | 0.2528 | 0.0032 | 0.2464 | 0.2591 |
| 1 | $\alpha_2$ | 0.0098 | 0.0003 | 0.0096 | 1.0065 |
| 1 | $n_2$ | 6.4965 | 0.3091 | 5.8903 | 7.1028 |
| 2 | $K_s$ | 2.3949 | 0.2876 | 1.8308 | 2.959 |
| 2 | $l$ | 2.4467 | 1.2516 | -0.0086 | 4.9019 |
| 2 | $w_2$ | 0.1261 | 0.0142 | 0.0982 | 0.1539 |
| 2 | $\alpha_2$ | 0.0081 | 0.0004 | 0.0073 | 0.0089 |
| 2 | $n_2$ | 3.7859 | 0.5376 | 2.7313 | 4.8406 |

| | | | | 95% Confidence limits | |
|---|---|---|---|---|---|
| Material | Variable | Value | S.E.Coeff. | Lower | Upper |
| 1 | $K_s$ | 0.1215 | 0.0009 | 0.1198 | 0.1233 |
| 1 | $w_2$ | 0.2849 | 0.0031 | 0.2788 | 0.2909 |
| 2 | $K_s$ | 1.7587 | 0.0676 | 1.6261 | 1.8913 |
| 2 | $w_2$ | 0.3467 | 0.0191 | 0.3092 | 0.3842 |

The Kernza model which only further optimized $K_s$ and $w_2$ was selected, because it had the lowest AIC score compared to models where more than these parameters were optimized ($\Delta$AIC>2). The fully optimized parameters were than used in a forward simulation of the root water uptake of Kernza (see next chapter).

## Root water uptake

The year 2018 proved to be dryer than the year 2019 (fig. 10). Temperatures in the spring rose earlier and reached a higher peak in 2018 than in 2019, which led to a higher evapotranspiration demand which could not be supplied by the limited rainfall, which led to a high precipitation deficit in the summer and fall of 2018. In 2018 almost no rain fell from May to July. Due to this precipitation deficit, the upper 75cm of the soil dried up and RWU shifted to the lower parts of the root system (fig. 11a). In the regions where root water uptake was allowed, it was highly correlated to the RLD. White areas offer insight into in which periods and soil depths root water uptake ceased.

In Hydrus Feddes water stress parameters were specified which determined the pressure head at which the soil becomes too dry (h ≤ -8000cm) or too wet (h ≥ -10cm) for the roots to take up water.

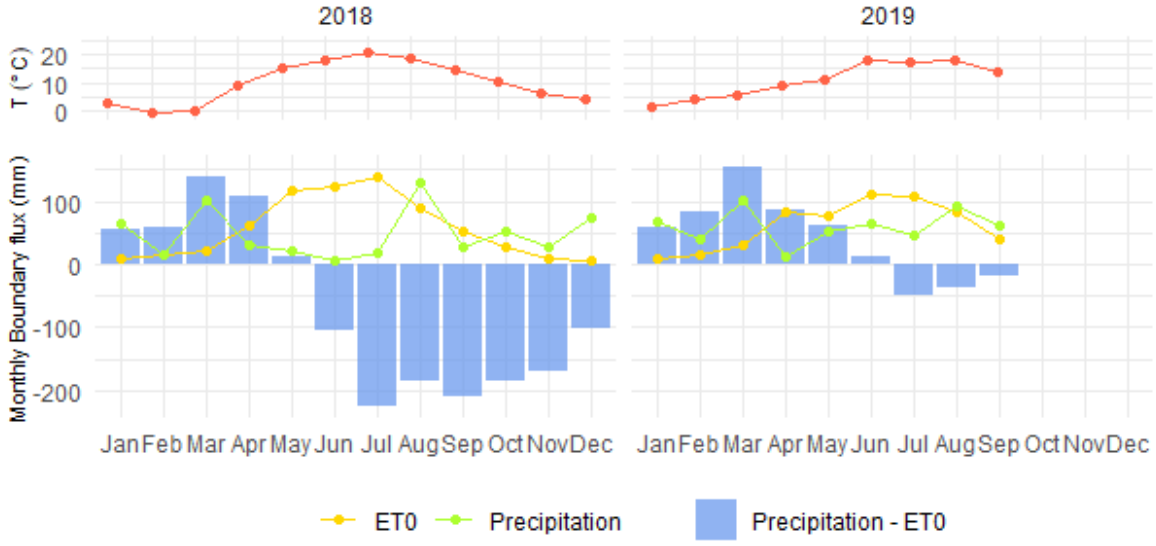


*Figure 10 The mean monthly temperature (°C), precipitation (mm) and reference evapotranspiration (mm) and the cumulative monthly difference between precipitation and $ET_0$ in the years 2018 and 2019. The window for 2019 presents values up to the 23rd of September.*

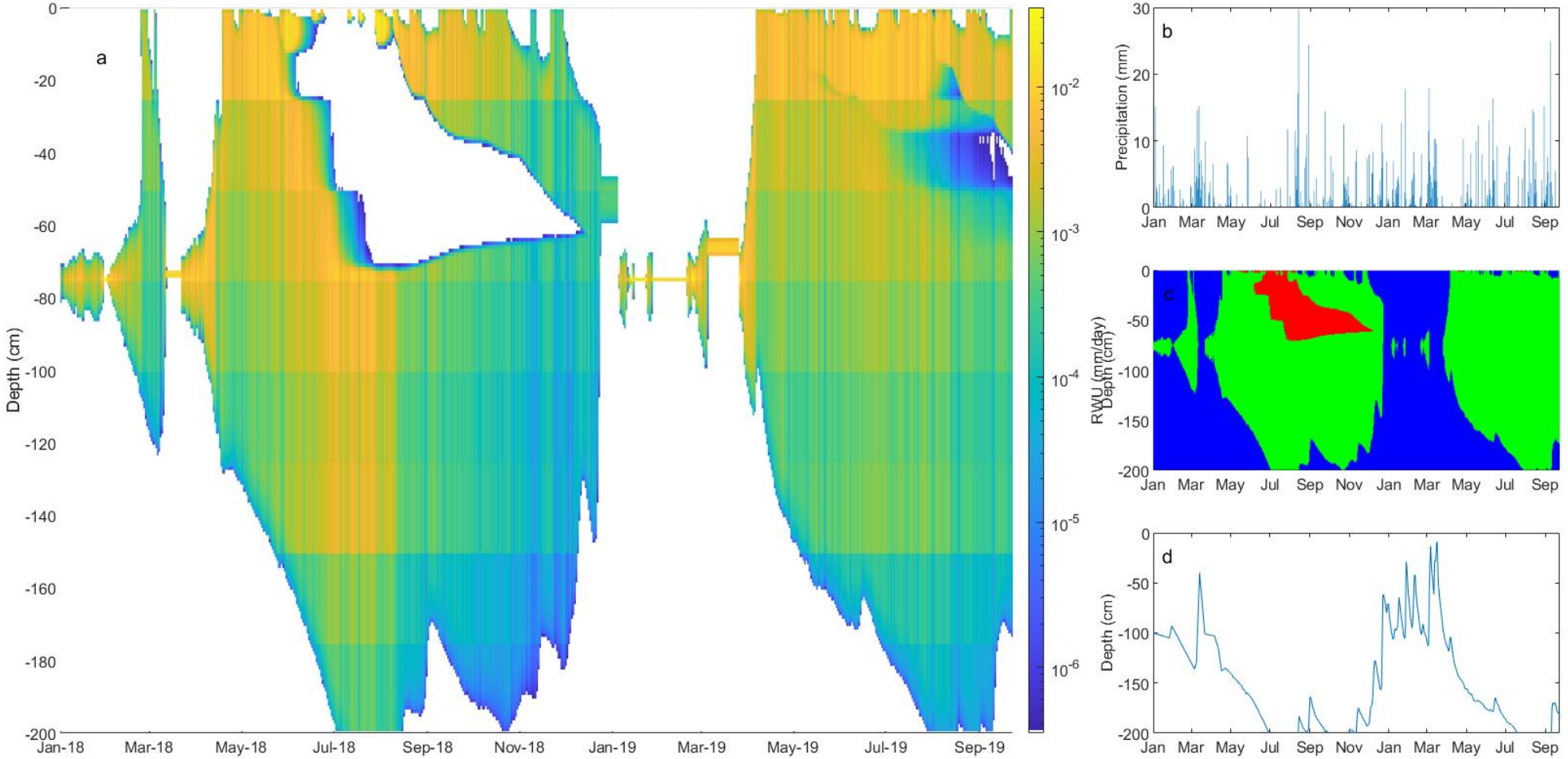


*Figure 11 Daily root water uptake (RWU) intensity per cm of soil depth (a), daily precipitation (b), daily pressure head value (c) and ground water level (d) during the modelled period. White regions in figure a represent the absence of root water uptake. Blue regions in figure c represent pressure head (h) values above -10cm (too wet for RWU), red regions represent h-values below -8000cm (too dry for RWU), green regions represent h-values between -10 and -8000cm where conditions allowed for root water uptake to take place.*

In Figure 11b pressure head values of ≤ -8000cm were colored red indicating that the soil was too dry for water to be taken up from it, values between -8000cm and -10cm were colored green indicating the presence of RWU and values ≥ -10cm were colored blue indicating the soil was wet enough to cause anoxia and stop water from being taken up by the roots in this region. Figure 11d shows that the absence of RWU in the bottom half of figure 11a can be mostly explained by the fluctuating groundwater table and figure 11b shows that a great deal of the absence of RWU in the top half of figure 11a is explained by the frequency and intensity of precipitation.

Most importantly, in the summer of 2018 a period without root water uptake in the upper 75cm of soil can be observed lasting from June to December. In June the soil in the first 75cm of soil became too dry due to water depletion and the absence of enough rainfall (fig 11b). In late August a few big rain events took place which replenished the soil from the top down until the soil throughout the first 75cm became wet enough again to allow for root water uptake. Transpiration demand also declined after summer which aided the repletion of the soil water. That the whole process of reestablishing root water uptake in the upper 75cm of soil took 6 months can be attributed to the low hydraulic conductivity of the soil in the top 75cm (table 5b), which caused water too only very slowly percolate to greater depths. In 2019 only a short period and a small portion of the soil profile lacked an adequate supply of water to allow for RWU to take place.

This resulted in a greater shift in regard to root water uptake to the deep roots in 2018 than in 2019 2019 (fig. 12). In the summer of 2019, the soil water storage was adequately filled with water, most water was taken up from the topsoil where Kernza had its highest root length density. The contribution of the deep roots remained small and stable throughout the growing season of 2019. In contrast in 2018 during the period between anthesis and harvest, indicated by the black dotted lines in figure 12, deep roots (≥1m deep) were responsible for almost 50% of the total root water uptake.

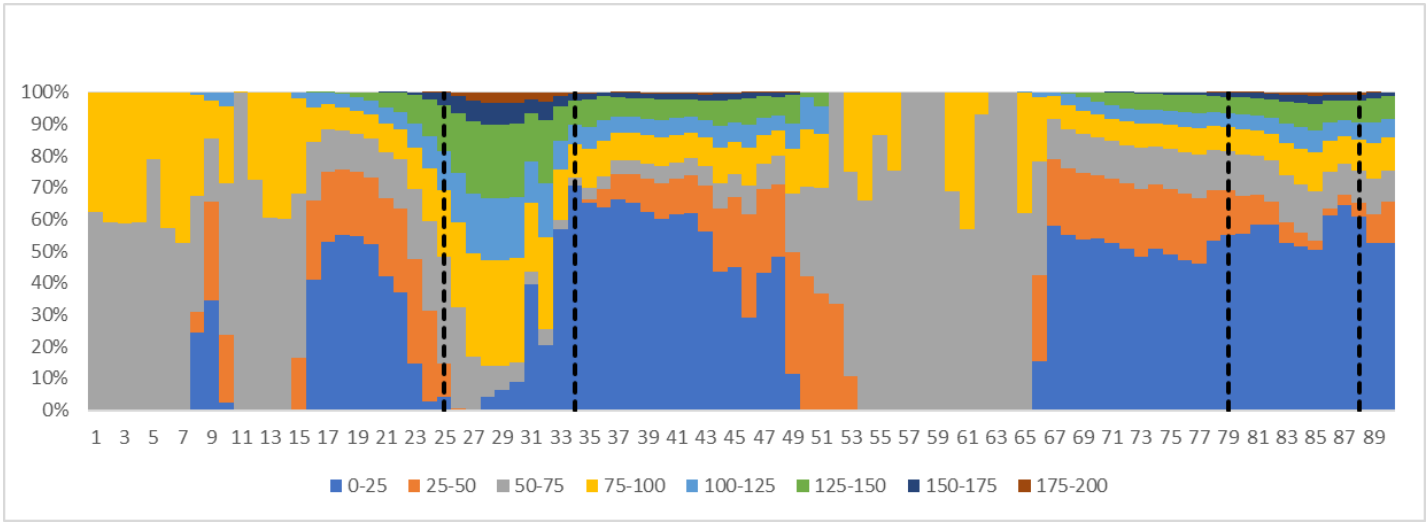


Figure 12 Weekly relative contribution of 25cm intervals to total root water uptake over the period from 1-1-2018 to 23-9-2019. Black dotted lines demarcate the period between anthesis and harvest.

# Discussion

## Isotope experiment

### Natural abundance of $^{18}O$ and $^{2}H$

Since leaves and stems were sampled and stored in the same test tubes, it had to be made certain whether mixing of water from these sources had occurred. This is important since there is no isotopic discrimination when soil water is taken up into the roots and neither during transport through the xylem, however isotopic fractionation does occur at the evaporative sites at the leaves' surface due to the difference in weight between isotopes (Flanagan & Ehleringer, 1991). Lighter isotopes require less energy to change phase during evaporation relative to heavier isotopes, which causes leaves to be relatively enriched in $^{2}H$ and $^{18}O$ compared to stem water. However from figure 3 it became clear that the stem and leaf water samples had similar delta values for both stable isotopes, which indicates that the water from these sources may have been mixed in the test tubes after sampling and storing them together despite the effort to separate leaves and stems from each other before analysis.

Moreover, the dual isotope plot of figure 4 provided further evidence for the occurrence of fractionation and mixing of leaf and stem water. The fractionation of $^{2}H$ and $^{18}O$ at the leaf surface occur at different rates. Leaves therefore have a $^{2}H/^{18}O$ ratio which differs from that of the soil water, visible in the slope of the regression lines in figure 4. The slope of the regression line of the stem samples also showed a deviation from soil water, albeit not significant. A trend however seems to be visible, since the slope of the regression line of the stem water samples seems to be intermediary between soil and leaf samples, which also pointed toward the mixing of stem and leaf water.

The most definite line of evidence came from figure 5. Plants take up water from the soil they are growing in, and do so without discrimination between isotopes, so the water found in the xylem in stems represent a mixture of the water taken up by all roots and should fall within range of the isotopic signature of the soil profile. Consequently, it is possible to use a multi-source mixing model, which takes into account the root length density (fig. 2) and volumetric water content (fig. 6) to calculate the probability with which water from each 25cm interval of the soil profile contributed to the water extracted from stem xylem. However, from figure 5a and b it became clear that the isotopic signature of the stems fell outside of the range of the soil profile. The stem mean delta values did not correspond to rainwater samples, but were identical to delta values of the ground water (fig. 3). However, taking the root length density into account again and with the ground water at a depth of about 185cm it is impossible for the plants to acquire all its root water uptake from groundwater. The analyzed natural abundance samples thus proved to be inadequate for determining the depth of root water uptake with a multi-source mixing model due to erroneous sampling method.

### Shortcomings of sampling methods

It is clear that leaves should not have been sampled, because their isotopic signature does not represent source water and that mixing of stem and leaf water occurs when sampled and stored in the same test tubes.

Moreover, the fact that the subject of this study was a herbaceous species adds another layer of uncertainty on top. Most studies of root water uptake involving the use of stable isotopes have been done on woody species. Woody species have clear separation of phloem and xylem. When xylem water needs to be sampled, a horizontal core is taken from the trunk after which the bark, where the phloem resides, is simply removed. What you are left with, then, is wood and unaltered source water. In herbaceous species phloem and xylem are not divided in this manner, in fact both types of vessels are in very close proximity of each other and are in most cases only a few cells in diameter. When sampling a stem, distinguishing between xylem, phloem water is not as easy, if not impossible. Phloem water, when flowing back from the leaves to the roots possibly becomes more enriched the more leaves it passes. Besides, herbaceous species have a green, and thus transpiring stem epidermis. This means that fractionation due to transpiration even occurs in small amounts along the stem.

One way to solve this would be to sample pure xylem water instead of plant organs, which is possible in herbaceous plants. To do so, one would cut of the stem close to the base and wrap a piece of parafilm around the wound forming a cylinder on top. Because of root pressure, water will be exuded from the cut and will accumulate in the parafilm cylinder where it can be collected from (Zegada-Lizarazu & Iijima, 2004). This water will have no interference from phloem water at all and will not have been subject to fractionation if stored immediately and correctly.

It is however questionable whether enough water could be acquired using this method on Kernza, since it has a hollow stem and only a small surface wherefrom water can be sampled. Windy situations in the field may also limit the applicability of this method. Therefor it seems that the best sampling technique would be to sample the root crown. The root crown lacks a green transpiring epidermis and resides just below the surface so that evaporation from the root crown is limited. Barnard et al. (2006) found the root crown to contain the water, best representative of source water, although basal stem samples also showed workable results. Root crown sampling could limit the number of samples that could have been taken around the injection PVC tubes due to its destructive nature, so that basal stem sampling becomes the preferred strategy.

Furthermore, transpiration water samples are also useless in quantifying the contribution of roots from different depths to the total root water uptake of a plant. From figure 3 it can be understood that transpiration water samples differ very strongly from stem samples. Due to the same process of evaporation at the leaf surface, transpiration water is expected to be relatively depleted in $^{2}H$ and $^{18}O$. However, transpiration water was shown in figure 3 to be enriched even in comparison to leaf water.

This unexpected result pointed towards the unreliability of this sampling method for the quantitative assessment of root water uptake with depth. The enrichment contrary to the expected depletion of transpiration water was likely caused by the sampling method itself. A bag was placed over a number of tillers to collect transpiration water, but atmospheric water vapor was also present in the interior of the plastic bag. This atmospheric water consequently also condensed on the inside of the bag. If the atmospheric water had an enriched signal, which was not measured, this could have contributed to the enrichment of the sampled transpiration water. Another factor which might have been at play, is that condensation is the reverse process of evaporation, fractionation thus also occurred in reverse fashion with the heavier isotopes condensing more easily than the lighter isotopes, leading to enrichment in the condensed transpiration water.

### $^{2}$H Tracer experiment

Both stem and transpiration water did not reflect source water and could therefore not be used in a mixing model to determine the depth of root water uptake, since their isotopic composition do not reflect the isotopic composition of the up taken water due to fractionation and mixing. This also holds true for the tracer experiment. However their δ-values can be interpreted as an on/off signal in the tracer experiment. The tracer used in the experiment was enriched with $^{2}$H to a delta value of 300000‰ in June and 600000‰ in July. Using this high of an enriched tracer means that every enriched signal that might be picked up can only be due to tracer uptake.

This enrichment showed up only in the transpiration water sample of day 16 after tracer injection in July. Although both transpiration and stem water samples were obtained from tillers growing directly around the PVC pipe, where the tracer was injected, transpiration and stem samples were not taken from the same tillers as both samples were taken simultaneously. As one plant may have deeper roots or a higher RLD at depth than another, tracer uptake may differ between plants. Therefore the enrichment showed up in one sampling type and not per definition also in the other on the same day. In July, the RLD at 1.75-2m deep in plot 4.1 (0.26) was higher than the RLD of plot 6.1 and 11.1 (0.07 and 0.04 respectively) which may have caused the relatively higher tracer uptake in plot 4.1 compared to the other plots.

Furthermore, the magnitude of enrichment was very small on this 16$^{th}$ day after injection in July, especially when the delta value of the tracer was taken into account. Four factors could have contributed to this separately or in every possible combination. These same factors may also have caused the tracer to not show up on the other sampling dates.

1) The RLD at 2m depth was very small. The RLD of the depth interval between 1.75 and 2.0m had an average RLD of 0.09 cm cm$^{-3}$. It is important to understand this value is a mean value for every cm between 1.75 and 2.0 depth. Although we are not sure of the exact RLD at 2.0 depth, it is likely to be smaller than the average of the mentioned interval and is only a fraction

of the total summed RLD. Water uptake is strongly related to RLD. The water in stem xylem or transpiration consists of a mixture of the water taken up by all roots. Since the water taken up by roots at 2m depth only contributes a very small amount to the total root water uptake, the tracer is mixed and diluted internally in the plant to a large extent. And therefore the lack of roots at 2m depth may have caused the effect of the tracer on the delta value of the sample to remain minimal.

2) Dilution in the soil matrix may have occurred. On the dates of tracer injection in both June and July, the water table in the ground water well closest to the plots was high enough to submerge the bottom of the PVC pipes (see fig. 8d). Thus, when the tracer was applied, it was diluted with relatively $^{2}H$-depleted groundwater that was present in abundance. The groundwater also caused anoxic condition for the roots at the depth of tracer injection so that water uptake by the roots at this depth was ceased, limiting tracer uptake.
3) The hydraulic conductivity of the soil is very low. The tracer may not have moved a lot from the PVC pipe into the soil and subsequently may not have reached many roots.
4) Destructiveness of the soil coring method. The soil drilling may have destroyed a fraction of the already small amount of roots present at 2m below the surface, lowering the capacity of water uptake from this depth.

## Modelling RWU in Hydrus 1D

Although Kernza was shown to rely mostly on its root in the top meter of the soil when precipitation was abundant, it was able to shift its water uptake to the bottom half of its root system when uppermost meter of the soil dried up. When surface droughts thus occur, and they will occur more often in the future due to climate change, these deep roots become of great importance in mitigating water stress and maintaining grain yields. This points towards the usefulness of Kernza in climate adaptive agriculture and food security in a changing climate.

This can be, however, very site and soil specific. In arid, rain fed systems water stores may be depleted by deep, perennial roots before the advent of flowering. Annuals grow their roots deeper during the growing season and as the upper soil layers dry up reach deeper hydrated soil layers. Perennials may have extended their root into these deeper layers from the onset of the growing season and also make use of the water present in these deeper layers from the start. Deep, perennial roots may thus risk depleting water sources before the stage of anthesis when these stores are needed. This problem may be exacerbated by the higher ET and longer growing seasons demonstrated by perennial crops such as Kernza in comparison to annual congeneric species (Oliveira et al., 2019; Sutherlin et al., 2019). Furthermore, drought tolerant annual cereals are able to initiate root growth when temporary

surface droughts occur. It is not uncommon for certain varieties of winter cereals to root and take water up from up to 2m deep (Thorup-Kristensen et al., 2009; Weir & Barraclough, 1986). The benefit of the perennial character of Kernza roots over the roots of drought tolerant annuals ceral crops thus remains unclear.

Similar studies in the future should apply drought treatments to assess the performance of Kernza under different drought conditions. Likewise, similar studies should be performed on deeper soils with deeper ground water tables and in more arid, marginal locations where Kernza could provide a regenerative and solution to falling productivity due to droughts and desertification and/or erosion. Performance of Kernza should be compared to drought tolerant varieties of annual cereals in all of these cases in order to provide farmers and policy makers with the appropriate information to decide whether perennial grains could improve their region's agricultural sustainability where annuals grain are nowadays grown.

### Model accuracy

The fact that only two retention curves were used to represent water retention in a 2.5m soil column and that inverse modelling was performed using only three TDR curves, present a limitation to the accuracy and precision of the model. Most of the fluctuations in water content happen in the top 25cm of the soil due to the RLD and evaporation influences being the highest in the topsoil. However, since retention data or water content fluctuation were not measured in the top soil, behavior of water in this part of the soil column relies solely on the estimation by the model. The lack of input for the inverse modelling may also be the cause of the low hydraulic conductivity prediction as seen in table 5b. It is questionable that the hydraulic conductivity in reality is that low in the range of 0-75cm depth. To improve the accuracy and precision of the model, retention and volumetric water content curves should have been measured at a greater resolution. The saturated hydraulic conductivity could also have been measured instead of inverse modelled.

## Yield and trade-off between perennation and seed production

Kernza grain yields still lack greatly behind its annual cereal cousins. Trade-off theory states that photosynthates and nutrients must be divided between sexual and asexual reproductive organs. The phenotypic trade-off theory fails to recognize that overall fitness may be enhanced by selection and while it may be true that a carbon atom cannot be in a rhizome or seed at the same time, an increased fitness may increase the total amount of C available to both structures (Tassel et al. (2010). Perennial grain crops such as Kernza have longer growing seasons, start photosynthesizing earlier in the season and maintain green tissue for longer, have a re-usable root system which is fully functioning when annual roots have not yet developed or have already senesced, and regrow and thus continue to photosynthesize after harvest. Perennial grain crops could pay for their additional costs of longevity

with these additional resources that are unavailable to the annual crop besides producing comparable grain yields with the resources captured in the period of the annual growth season (Cox et al., 2010; DeHaan et al., 2005; Tassel et al., 2010). A gram-for-gram trade-off therefore should only exist in the first year after sowing, when the plant is building it perennating structures.

Low yields may not be caused by an inherent physiological constraints, but natural selection which selected for longevity in perennials (DeHaan et al., 2005). Natural selection in the ancient agricultural landscape favored fast growing, seed dispersing annuals that could cope with the periodic disturbance brought about by introduction of the plow and burning methods used by early agricultural societies. High yielding perennial grain crops could not have evolved by natural or unconscious selection in this environment (Tassel et al., 2010). Artificial selection under condition of chemical fertilization and weed and disease control further increased the reproductive allocation of annuals. Perennial grain breeding have received only a fraction of the attention and resources compared to annuals. The first breeding programs for a perennial cereal only started in the 1987 (Wagoner et al., 1990) and were initially only performed by The Rodale Institute and later exclusively by The Land Institute with limited resources compared to conventional breeding companies. Kernza has only been commercially available since 2013. The domestication of annual cereals on the other hand goes back some 8000-10000 years with big investments in breeding being made over the last century. Breeding of perennial grain will not take as long, as perennial species can be hybridized with related annuals to incorporate domestication traits more quickly and we can make use of genetic markers nowadays (Cox et al., 2010).

As it happens, Kernza yield have been increased by breeding. Kernza has undergone a total of 8 selection cycles in about two decades. The first two were performed at the Rodale Institute by Wagoner et al. (2008). The other six were performed at the Land Institute in the last 14 years, the last one in the fall of 2015. Wagoner et al. (2008) were able to achieve yield increases of 20% per cycle. After two cycles at the Land Institute yield per area and mass per seed were increased by 77 and 23% respectively. Accumulated average gains from selection over 5 cycles was 143% for grain yield per head and 60% for mass per kernel (DeHaan et al., 2018). At the Land Institute selection at first focused on improving yield an later on improving other traits necessary for production in an agricultural setting. In cycle four when population size was large and the number of selection traits limited to three to improve yield, 40.5% in seed yield per head were achieved. In cycle six when selection was performed on 13 traits to improve agronomic use, the yield increase per head was limited to 15% (DeHaan et al., 2018).

*Table 6 Grain yield, forage yield, harvest index (HI) and thousand kernel weight (TKW) obtained from the experiment described in this thesis.*

| Yield (t/ha) | Forage yield (t/ha) | HI | TKW (g) | Number of grains per $m^2$ |
|---|---|---|---|---|
| 0.95 | 13.834 | 0.06 | 5.4 | 17525 |

Through breeding and management improvements yields have gone from 280-679kg $ha^{-1}$ (Wagoner et al., 1990) to the maximum yield found in the literature of 1662 kg $ha^{-1}$ (Culman et al., 2013) although more typical yields are comparable to the one obtained in our study, which is displayed in table 6. Wheat yields in Denmark on average around six tons per hectare in the period from 1992 to 2008 (Kristensen et al., 2011). Kernza yield in our study thus represent only 1/6 of the national average conventional wheat yield. Reaching competitive yield levels may still take multiple decades and may be first reached under marginal conditions (Cox et al., 2010). Ultimately the question whether competitively yielding perennials grain crops can be developed can only be answered empirically by trying (DeHaan et al., 2018).

## Multifunctional management

Although Kernza yields are projected to increase through genetic improvement in the coming decades, agronomic improvements could make up for low yields today and increase the competitiveness of Kernza relative to annual grains. A greater investment in the development of optimal farming practices and strategies is needed to convince farmers to plant and experiment with Kernza (Adebiyi et al., 2015). To counterbalance low grain yields, Kernza should be deployed as a multifunctional crop, that is, a crop that besides providing grains is also used to provide other sources of income and ecosystem services (Ryan et al., 2018). This holds especially true for the present, while grain yields are still too low to be profitable in and of itself, but also in future years when sufficient grain yields cannot be reached due to yield-limiting circumstances. Multifunctionality will however still be a key benefit of Kernza in future high grain yielding scenarios.

### Grazing and crop residue

Contrary to grain yields, overall biomass yields of Kernza are high and more stable, reaching up to 17 ton/ha (Culman et al., 2013; Jungers et al., 2019; Tautges et al., 2019), which has led to the idea of using Kernza as a dual-purpose crop, meaning that Kernza could be harvested for both grain and straw (Ryan et al., 2018).

The perennial growth habits of Kernza give rise to three windows of opportunity each year for harvesting or grazing upon the vegetative biomass. The early season growth before the phase of culm elongation and the late season post-harvest regrowth can be grazed by livestock. Thirdly, the crop residue after summer harvest can be used as fodder and the remaining stubble could be grazed on as

well (Ryan et al., 2018).

Favre et al. (2019) calculated for these three stages the nutritional value. These values are similar or greater to forage IWG and other common cool-season forage grasses in the Upper Midwestern United States and suitable to feed to livestock (Favre et al., 2019). One advantage of IWG straw is that at the time when the grains are ready for harvest, the stems remain green for the greatest part, resulting in a high N content (Hayes et al., 2012). This adds nutritional value to the straw and gives it an nutritional advantage over annual grain straw. Kernza crop residue has been shown to have a crude protein content which is 30% higher than wheat straw (Favre et al., 2019).

Harvesting or grazing early and late season forage have been shown to increase root biomass, root turnover and nitrogen cycling in comparison to harvesting for grain only, thereby increasing forage and grain yields (Pugliese et al., 2019). They hypothesize that harvesting for forage drives increases in root exudation and turnover which increases decomposition rates to free up nutrients for above-ground regrowth.

Bell (2013) reported that profits derived from Kernza may be 38% higher when grazing is applied alongside grain harvest, relative to harvesting grains only.

### Intercropping and perennial polycultures

Other ways to improve the utility and productivity of Kernza is by incorporating it into intercropping or polyculture systems. Kernza may provide benefits to neighboring crops and conversely neighboring crops may provide benefits to Kernza. Declining yields as stands age may be due to nutrient depletion but N fertilization may reduce sustainability of perennial grain production, so intercropping may be a useful ways to improve nutrient availability in aging Kernza stands (Tautges et al., 2018). Intercropping may increase investment in sexual reproduction by increasing below-ground competition, thereby increasing grain yield. Intercropping could also increase N-input through biological fixation, increase P-solubilization and stimulate microbial activity and soil organic matter mineralization thereby increasing the availability of nutrients (Tautges et al., 2018).

Weik et al. (2002) found out that it might be difficult to intercrop perennial grain crops because of asynchronous seed maturation. To circumvent the problem of seed shattering in asynchronous perennial grain legumes, forage legume crops may be better suited for growing in mixed stands or intercropping with IWG. Another advantage of forage over grain legumes may be that less export of N is exported off the field (Hayes et al., 2017).

But an additional problem is maintenance of crop abundance. According to Tautges et al. (2018) effectivity of intercropping may differ greatly among sites and climatic conditions and is dependent on intercrop establishment. Some legume intercrops like alfalfa can be quite competitive in terms of soil nutrient and water uptake, and may cause problems to Kernza stand maintenance over

the years (Dick et al., 2018; Tautges et al., 2018). Li et al. (2019) therefore advises moderate fertilizer application to reduce competitive advantage of alfalfa and keep in from dominating Kernza.

Other studies suggest Kernza may be to competitive when grown in mixed stands with sweet or subterranean clover but found white clover to perform fine alongside Kernza (Hayes et al., 2017; Dick et al. 2018). Hayes et al. (2017) showed that subterranean clover could compensate in terms of N-fixation for the annual removal of 1.5-2.0 tons of grain per hectare.

Longer trials are needed (>5 years). The yield decline common after the second year and the influence of legume intercropping on yield persistence are often not in investigated, although this is one of the biggest problems faced by perennial grain crops, and one of the main reasons for including legume intercrops (Pugliese et al., 2019; Vico, 2016).

Tautges et al., (2018) performed such a longer term study, which included the critical 3$^{rd}$ and 4$^{th}$ year by intercropping alfalfa with Kernza. And found that from year 3 to 4 yield were kept relatively stable in bi-cultures whereas they showed stronger declines in both unfertilized and fertilized monocultures. Alfalfa biomass was found to be positively correlated with grain yield, nutrient uptake and harvest index of Kernza in the fourth year of the study. The positive influence of alfalfa intercropping on the Kernza HI means that the investment in seed relative to vegetative biomass may be improved by intercropping. This study, in contrast to Hayes et al. (2017), suggests that N fertilization may be needed anyway in later years to supply adequate amounts of N. Effects on N were shown to be initially absent but cumulative over the years (Li et al., 2019). An interesting management strategy that should be investigated would be to sow Kernza in two or three year old alfalfa stands, where N has had the time to accumulate through organic matter.

Future studies to should investigated to what extent deep roots of Kernza could escape competition for water with intercrops.

### Crop rotations

Surprisingly, only one study to this day has directly studied how and how fast Kernza could improve ecosystem services compared to an annual wheat crop (Culman et al., 2013). This two-year study therefore serves as an indicator of how useful Kernza could be in improving (per unit input) productivity of a crop rotation, which points too another facet of the multifunctionality of this perennial crop.

Kernza showed to have lower soil moisture levels in the deeper part of the soil, which may indicate less drainage or a higher root water uptake at depth (Culman et al., 2013). In the second year of the study by Culman et al. (2013) Kernza showed lower soil moisture levels in the top soil compared to wheat. The authors hypothesize the latter may be due to greater evaporative losses in annual wheat due to a less developed canopy or from increased root turnover causing the formation of macropores

and channels which increased rainwater infiltration (Culman et al., 2013).

Furthermore, in the first year $NO_3$ leaching was not influenced by crop type, but in the second year Kernza showed low to almost negligible concentrations of $NO_3$ below the rooting zone whereas at the same depth below wheat significantly higher nitrate levels were detected in comparison to the perennial Kernza and in comparison to the previous year below wheat (Culman et al., 2013). Kernza thus reduces leaching in the second year, whereas leaching was increased under wheat in the second year. Similar results were found by Jungers et al. (2019) when Kernza was compared to maize. The higher C mineralization rates in soils under Kernza furthermore suggest that over a two-year period more biologically active carbon is present under Kernza relative to annual wheat (Culman et al., 2013). Kernza would thus be suitable crop to improve annual crop rotation, a problem that would have to be solved in the termination of the perennial crop and the influence of this management choice on the previously provided ecosystem benefits. If other high yielding perennial grains will be bred, crop rotations could possibly rotate from perennial crop to perennial crop (Ryan et al., 2018).

# Conclusion

Kernza shows the ability reach and maintain their deep root biomass throughout the year and thereby reach deep water stores. In periods of surface drought this allows them to shift their root water uptake to deeper soil layers where there is still sufficient water. This may contribute to yield stability in an increasingly variable climate with where periods with rainfall surpluses alternate with periodic droughts. However, the extent of the benefits in terms of drought tolerance of Kernza over deep rooting and drought tolerant annual cereals remains unclear. Kernza yields and yield stability are also generally still lower than those of annual cereals but this can be improved by breeding. Multifunctional use of Kernza can also increase agroecosystem productivity to offset low grain yields. When yield and yield stability have been improved through breeding, the deep roots of Kernza may provide an important benefit to maintaining these yield in periods of drought, where annual grain crops may fail to do so due to a lack of deep roots.

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