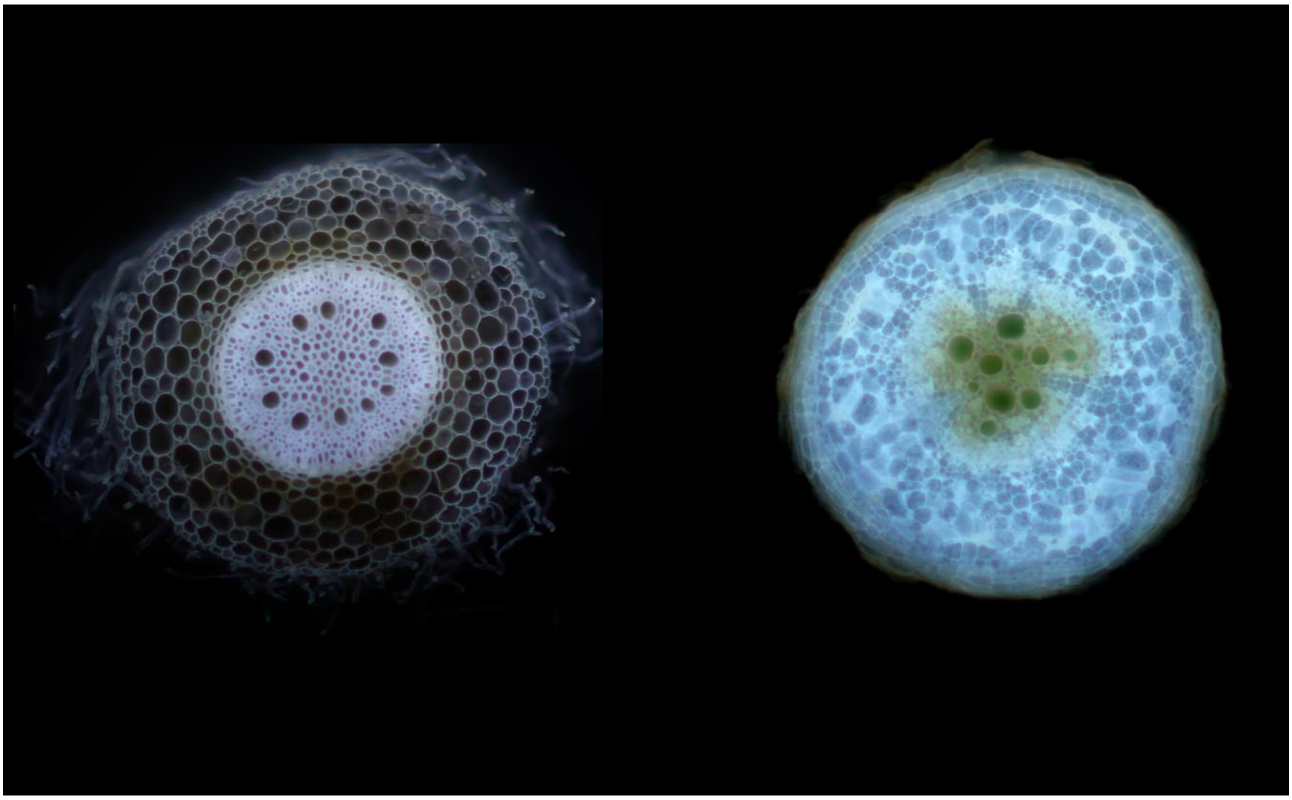
UNIVERSITY OF COPENHAGEN
FACULTY OR DEPARTMENT



PhD Thesis

Corentin Bonaventure Lucien Roger CLÉMENT

Deep water uptake of perennial crops

A case study on intermediate wheatgrass and alfalfa.

Supervisor: Dorte Bodin Dresbøll and Kristian Thorup-Kristensen

Submitted on: 06 February 2021

Name of department:	Section for crop sciences, Department of Plant and Environmental Sciences Faculty of Science, University of Copenhagen, Denmark.
Author(s):	Corentin Bonaventure Lucien Roger CLÉMENT
Title and subtitle:	Deep water uptake of perennial crops. A case study on intermediate wheatgrass and alfalfa.
Topic description:	Deep water uptake by perennial crops
Supervisor:	Dorte Bodin Dresbøll and Kristian Thorup-Kristensen
Submitted on:	06 February 2021

Front Page Photo. Root radial cross-section of intermediate wheatgrass (left) and alfalfa (right). Scale is not respected.

Summary [English]

The perfect storm. That is the term used by Gerald C. Nelson to describe the triple challenge of increasing food production while adapting to climate change and reducing the environmental impact of agricultural systems. Nowadays, conventional farming systems are showing some limitations, such as low resources use efficiency and poor ecosystems services that appear to be associated to the loss of plant diversity and perenniality in crop rotations. In addition, water, the most important yield limiting factor worldwide, will increasingly restrict food production in the future due to rainfall shortage and increase in human consumption. In such context, perennial crops, with denser and deeper root system could use resources in deep soil layers that are logically inaccessible to crops with shallower root system. The goal of this thesis was therefore to investigate the root growth and water uptake capacity of intermediate wheatgrass (Kernza®) and alfalfa, two deep rooted perennial crops, under field conditions and at great soil depth (i.e. 1.0-2.5 m).

Maintaining hydraulic continuity along the soil-plant-atmosphere continuum is a prerequisite for deep water uptake. At the plant level, hydraulic conductivity depends on complex anatomical and physiological processes among which the root system constitutes the second largest resistance to water flow. Therefore, in depth characterisation of root and xylem anatomy was done to understand the hydraulic properties of the crop root systems, with a focus on their evolution with soil depth. Crops were grown in the field, rhizoboxes, mesocosms and solution culture to take into account the variability of root type and soil depth as well as growing environment. For both crops, axial hydraulic conductance decreased with soil depth and along individual root segment. Alfalfa roots had greater axial hydraulic conductance in comparison to intermediate wheatgrass roots, especially at depth. Root and xylem anatomy were highly variable across crop species, root types and growing environments. In parallel, a combination of imaging and sensor technology, stable isotope techniques and a modelling approach was used to study root growth and water uptake under field conditions during the 2018-2019 seasons. Both crops presented roots down to 2.0 m soil depth that were active in terms of water uptake. Alfalfa had greater root length at depth and absorbed twice as much water below 1 m soil depth, than intermediate wheatgrass. For both crops, model simulations predicted that water uptake in deep soil layers (i.e. 1.5 – 2.0 m) increase (i.e.>30%) under dry conditions.

This thesis brings insights into the understudied field of root growth and water uptake at great soil depth. Particular efforts were put in understanding the environmental and agricultural contexts in which deep root growth, deep water uptake and the development of perennial cropping systems would be possible and favourable.

Summary [Danish]

Den perfekte storm. Dette begreb brugte Gerald C. Nelson til at beskrive den trefoldige udfordring det er at øge fødevareproduktionen, samtidig med at den skal tilpasses til klimaforandringerne og reducere miljøpåvirkningerne. Vores nuværende landbrugssystemer viser begrænsninger i forhold til en effektiv udnyttelse af ressourcer og økosystemstjenester, hvilket kan være forbundet med et fald i biodiversitet samt færre flerårige planter i dyrkningssystemerne. Vand er den vigtigste udbyttebegrænsende faktor globalt, og vil i stigende grad begrænse afgrødeproduktionen i takt med en forventet mindre nedbørsmængde og højere vandforbrug blandt mennesker. I den kontekst vil flerårige afgrøder, med større og dybere rodvækst, bedre kunne udnytte ressourcerne i de dybe jordlag, sammenlignet med afgrøder med mindre dybe rødder. Målet med denne afhandling var derfor at undersøge rodvækst og vandoptag, i dybe jordlag (dvs. 1.0-2.5 m) i marken, i lucerne og Kernza®, to flerårige afgrøder med dyb rodvækst.

At opretholde hydraulisk kontinuitet gennem jord-plante-atmosfære kontinuummet er en forudsætning for vandoptag fra dybe jordlag. Hydraulisk kontinuitet er afhængig af komplekse anatomiske og fysiologiske processer i planterne, hvori rodsystemet udgør den næststørste modstand i forhold til vandoptagelse. Der blev derfor udført en grundig karakterisering af rod- og xylemanatomi, for at forstå rodsystemernes hydrauliske egenskaber med fokus på udviklingen af dybe rødder. Afgrøderne blev dyrket i marken, i store rodkasser, i rør og i vandkultur for at tage forbehold for variabilitet af rodtype, jorddybde og vækstmiljø. For begge afgrøder aftog den aksiale hydrauliske ledningsevne med jorddybden og langs de individuelle rodsegmenter. Lucerne-rødderne havde større hydraulisk ledningsevne end Kernza®-rødderne, især ved større dybder. Rod- og xylemanatomi varierede på tværs af afgrøder, rodtyper og vækstforhold. Parallelt med anatomistudierne, blev rodvækst og vandoptag undersøgt i markforsøg i vækstsæsonerne 2018-2019, vha. billedtagning, sensorteknologi, brug af stabile isotoper og modellering. Begge afgrøder havde aktivt vandoptag ned til 2 meters dybde. Lucerne havde større rodlængde i de dybe jordlag og havde et dobbelt så stort vandoptag under 1 meter, sammenlignet med Kernza®. For begge afgrøder viste modelsimuleringer at vandoptag fra dybe jordlag (dvs. 1.5 – 2.0 m) øges (>30%) under tørre forhold.

.

Denne afhandling giver større indsigt i det understuderede felt omkring rodvækst og vandoptag i dybe jordlag. En særlig indsats blev lagt i at forstå under hvilke miljø- og landbrugsmæssige forhold dyb rodvækst, vandoptag i dybe jordlag og udvikling af flerårige afgrødesystemer, ville være mulige og favorable.

Summary [French]

La tempête parfaite. C'est le terme utilisé par Gerald C. Nelson pour décrire le triple défi qui consiste à augmenter la production alimentaire tout en s'adaptant au changement climatique mais en réduisant l'impact environnemental des systèmes agricoles. Aujourd'hui, les systèmes agricoles conventionnels présentent certaines limites, en terme d'efficacité et de services écosystémiques, qui semblent être associés au manque de diversité et de pérennité dans les rotations culturales. D'autres part, l'eau, le principal facteur limitant les rendements mondialement, limitera de plus en plus la production alimentaire dans les années à venir en raison du manque de précipitations et de l'augmentation de la consommation humaine. Dans ce contexte, les cultures pérennes, grâce à leurs systèmes racinaires plus denses et plus profonds, peuvent utiliser des ressources situés dans les couches profondes du sol qui sont logiquement inaccessibles aux cultures ayant un système racinaire plus superficiel. L'objectif de cette thèse est d'étudier au champ et à grande profondeur (entre 1.0 et 2.5 m), la croissance racinaire et la capacité d'absorption d'eau de l'agropyre intermédiaire (Kernza®) et de la luzerne, deux cultures pérennes à enracinement profond.

Le maintien de la continuité hydraulique le long du continuum sol-plante-atmosphère est une condition préalable à l'absorption d'eau en profondeur. Au sein de la plante, la conductivité hydraulique dépend de processus anatomiques et physiologiques complexes parmi lesquels le système racinaire représente la deuxième plus grande résistance à l'écoulement de l'eau. Une caractérisation approfondie de l'anatomie des racines et du xylème a donc été effectuée pour comprendre les propriétés hydrauliques des systèmes racinaires, en mettant l'accent sur leur évolution avec la profondeur de sol. Pour chaque culture, la conductance hydraulique axiale diminue en fonction de la profondeur de sol. Les racines de luzerne ont une conductance hydraulique axiale plus importante par rapport aux racines d'agropyre intermédiaire, surtout en profondeur. En parallèle, nous avons utilisé diverses méthodes (imagerie, capteurs, isotopes stables et modélisation) pour étudier la croissance racinaire et l'absorption d'eau de ces deux cultures durant les saisons 2018-2019. Les racines de ces deux cultures absorbent de l'eau jusqu'à 2.0 m de profondeur. La luzerne a plus de racine en profondeur et absorbe deux fois plus d'eau en dessous de 1 m de profondeur que l'agropyre intermédiaire. Pour les deux cultures, les prévisions du modèle ont montré que l'absorption d'eau dans les couches profondes du sol (i.e. 1.5 – 2.0 m) augmente (i.e. >30 %) en conditions de sècheresses.

Cette thèse apporte des connaissances sur la croissance des racines et de l'absorption d'eau à grande profondeur de sol, deux domaines peu étudiés. Un effort particulier a été déployé pour comprendre les contextes environnementaux et agricoles dans lesquels la croissance des racines profondes, l'absorption d'eau en profondeur et le développement de systèmes de cultures pérennes seraient possibles et bénéfique.

Acknowledgment

Firstly, I would like to thank my principal supervisor Kristian Thorup-Kristensen for leading this audacious research project and for giving me the opportunity to be part of it. I am sincerely glad to have done this PhD with you, thank you for letting me lead this project, for all the scientific discussions and for trusting me. I have gain a lot professionally and personally from your comments, advice and views on research and agricultural systems.

Secondly, I would like to thank my secondary supervisor, Dorte Bodin Dresbøll for keeping me motivated, helping in coordinating the work and completing very well with Kristian in the supervision tasks. Thank you for all your time, scientific discussions and your humble attitude which definitely helped me to maintain a healthy work/life balance.

Kristian, Dorte, I feel lucky to have been your student. Thank you for all the fruitful discussions and support you have given me during this project. I will bring home the nice relationship that we have built, the professionalism and the mixt of ambition and humility that Danes know how to combine well ☺. Thank you for giving me a lot of freedom and autonomy in my PhD. The DeepFrontier project made this possible, but I still think it takes a lot of open-mindedness to let a young researcher explore his own research ideas. For this especially, I am very grateful. Ultimately, thank you for reading my PhD thesis and giving me relevant feedback.

This doctoral work would not have been the same without the welcoming of Johnathan P. Lynch at the Penn State Root lab and the help of Hannah M. Schneider who has facilitated and been involved in all stages of my exchange study. Thank you both for all the scientific discussions, for your friendly and welcoming attitude during these five months and for your support throughout the process of my work. Working in another research group has been a real learning experience for me and building "root" bridges over the globe was particularly enjoyable. Thank you to all the members of the Penn State Root Lab, especially Jirawat Salungyu, James Burridge, Jagdeep Singh Sidhu, Jeena Reeger, Molly Hanlon, Christopher F. Strock, Peter Ilhardt, Robert Snyder and Kathleen M. Brown for welcoming me, for supporting me with the work and simply for being good friends.

I am particularly thankful to Nicolas Angeli, Loic Louis and Damien Bonal from the SilvaTech Platform for collaborating with me and for their very professional work and all the fruitful discussions we had during my brief stay.

I would also like to thank Fulai Liu and Eva Rosenqvist for allowing me to use some of their material, but especially for giving me advice and tips throughout the project. I'm sure you didn't realize it, but it had some influence on my work.

I am very grateful to the entire technical team of the Taastrup experimental farm for their skills and assistance in the greenhouse and in the field. I could not have done all this work without assistance from Anders Kristian Nørgaard, Allan Esben Hansen and Jason Allen Teem.

I would also like to thanks Tomke Susanne Wacker for the support she gave me all along this project, for all the good times and for the friendship that we have built. I hope I have been as nice and supportive as you have been.

This PhD project would not have been the same without the presence of Simon Fiil Svane. I cannot believe I am writing that but thank you for your scepticism and challenging discussions it helped me grow as a scientist and taught me how to stay calm. Overall, thank you for your friendship, all the good times and making me discover the true Denmark (i.e. Jutland? ☺).

I particularly thanks Niels Alvin Faircloth Olsen for always being so joyful, keeping me grounded, and for telling me all kinds of story about Danish history and mythology. After all, thank you for translating the abstract of this thesis in Danish.

I would also like to send a warm thank to Suzanne David for her joy, support and help with so many administrative tasks. You made my work much easier and I probably made yours worse ☺. Even more you made me forget about those grey days in Denmark which was very pleasant.

At last but not least, a great thanks to all the colleagues from the "root group" and the campus in Taastrup with which I had the pleasure to work and share moments during these three years. You have made my working environment very pleasant and I will take home many good memories.

I am particularly grateful to Joost Sleiderink and Kyriaki Adelais Boulata for being the first master students under my supervision. Both of you, be aware that your master's internships have been very enriching both professionally and personally for me.

I can only think when writing this acknowledgment section of all the encounters that lead me to doing this PhD in Denmark. I am particularly thankful to Alain Pierret, Jean-luc Maeght, Paul Pavellic and Hernán Ojeda for sowing the seeds of this research achievement and for sharing their vision of science.

I would like to thank all the housemates I have lived with during those three years, especially Hannah Linnemann which has supported me all along the process of this PhD.

Finally I would like to thanks all those I have not yet mentioned, who are also responsible for very good moments during the past three years:

- To all my friends and the others who have made my stay in Copenhagen very pleasant Baptiste, Søren, Else and the VUF crew.
- To all my friends and family members who kept me sane in this process and made me remember that a PhD is just a PhD.
- A particular thank you to Audrey Oranger for supporting me in so many aspects, for her sharp eye and for always managing to put me back on the track.

Anyway, my deepest apologies to all those I may have forgotten here…

Research rationale

It is recognized that global agriculture is facing an unprecedented challenge in increasing agricultural production under a context of worldwide demographic increase and climate change (Long and Ort 2010; Hunter et al. 2017). To meet human demand, it is estimated that agricultural production will need to increase by 25-75% by 2050 (Hunter et al. 2017). Predicted yield increase for common annual crops seems to be insufficient to cover such an increase in food demand (Ray et al. 2013). Furthermore, high-input agricultural systems that emerged after the green revolution are now showing their limitations in terms of productivity and environmental impacts. Building more efficient and resilient agricultural systems seems the only long-term sustainable way to improve food security worldwide without harming the environment or threatening public health (Tilman et al. 2002; Hunter et al. 2017; Weiner 2017). Therefore, there is an urgent need to investigate alternative crops and farming concepts that could address both production and environmental challenges.

Water, the most important yield-limiting factor globally, is regionally scarce, and scarcity is predicted to increase due to climate change and increasing human consumption, reaching an increase of 20% by 2050 (Rosegrant et al. 2009; Kummu et al. 2016). In this context, the number and severity of drought events will increase worldwide, affecting both rainfed- and irrigated-agricultural systems (Rosegrant et al. 2009; Turral et al. 2011). Improving soil resource acquisition and its physiological utilization seems to be two essential strategies to jointly increase crop nutrient and water use efficiency and maximize agricultural productivity in both high- and low-input agricultural systems (Lynch 2007a; Den Herder et al. 2010; Lynch and Brown 2012; Bishopp and Lynch 2015; Hamidov et al. 2018). Part of this challenge is to find adaptation strategies that will increase the efficiency and resilience of agricultural systems regarding water usage.

While agricultural systems are dominated by annual crops, perennial crops offer interesting prospects due to their deep and extensive root system associated to their longer growing period. They also ensure year-round ground cover and are supposed to reduce agricultural inputs, soil disturbances and to be more resilient to climate variability, compared to annual crops (Cox et al. 2006; Crews and Dehaan 2015). With such characteristics, they are now drawing more attention as they could address some of the above-mentioned challenges that modern agriculture is facing. With 80% of human diet being comprised of cereals and oil seed crops, the development of perennial grain crops is essential for the transition toward perennial agricultural systems. (FAO 2013). However, studies on perennial crops are usually restricted to studies on forage and native grassland which limits our understanding of their potential in terms of agricultural productivity and in achieving the above mentioned ecosystems benefits.

The potential for having a deep and profuse root system is one of the traits that make perennial crops particularly attracting. Increasing water uptake from deep soil layers seems to be an advantageous strategy in regions where water is available at depth. Such approach has proved to give improved crop productivity under drought in the case of wheat (Manschadi et al. 2006; Kirkegaard et al. 2007), rice (Fukai and Cooper 1995) and maize (Kondo et al. 2000), for example. However, studies from Prechsl et al. (2015) and Rasmussen et al. (2019) remind us that root presence in deep soil layers is not the sole drivers behind deep water uptake. In fact, the processes behind root growth and the functionality of roots in deep soil layers is not straightforward, especially due to the great environmental variability and the large range of plant responses to such variability (e.g. climate and soil) (Hamblin and Tennant 1987). Our understanding of root systems and rhizosphere (i.e. the soil influenced by plant roots) processes have received little attention from the scientific community, agronomists and plant breeders (Fig. 1). This knowledge gap is even greater when focusing at great soil depths (i.e. >1 m) as most research studies have focused on topsoil layers (Pierret et al. 2016). The study of deep rooted perennial crops like alfalfa and intermediate wheatgrass seek to address both knowledge gaps by gathering physiological knowledge on deep root functioning while studying the potential of perennial crops.

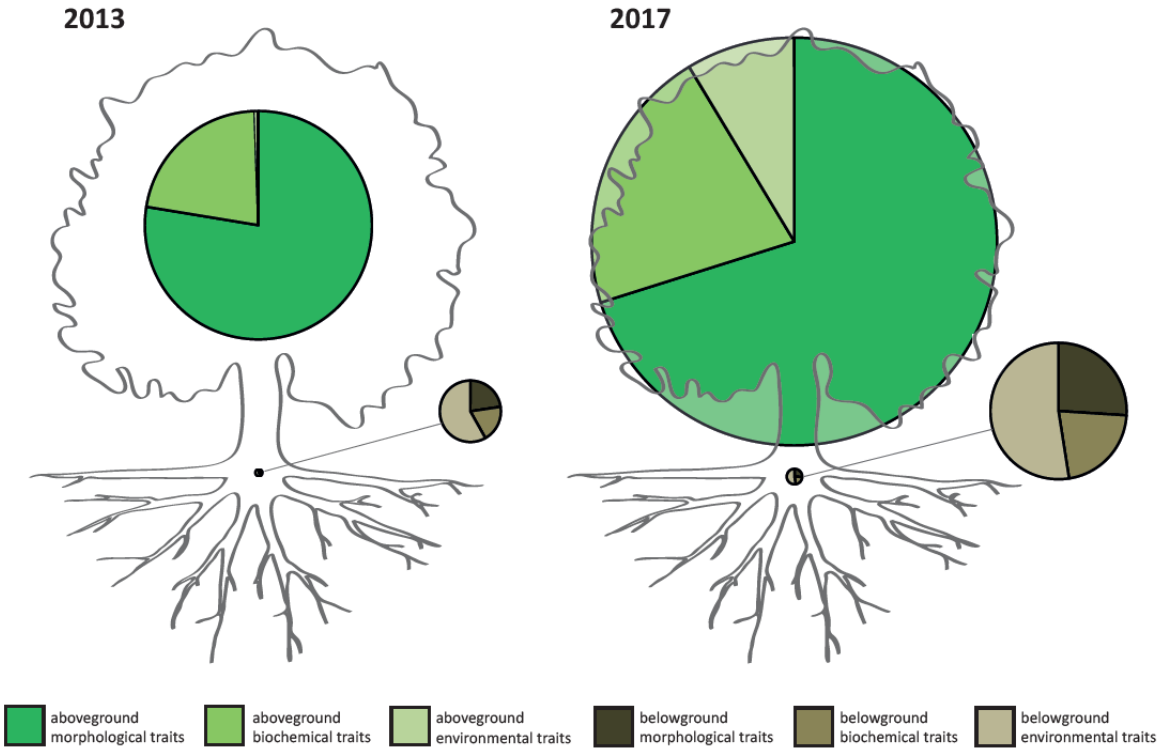


***Figure 1.* Proportion of root and shoot traits found in the TRY plant trait database (Kattge 2020) on the 18/08/2013 and 23/02/2017 from (Bergmann 2017). Size of the pie chart are proportional to each other**

Research objectives

The goal of this PhD was to study the capacity of intermediate wheatgrass, a promising perennial grain crop, and alfalfa, a perennial forage crop, to use water from deep soil layers (i.e. >1 m) under field conditions. Due to its deep root system and its capacity to use deep soil water (McCallum et al. 2001), alfalfa was chosen as a comparison crop to the less studied intermediate wheatgrass. Emphasis was placed on understanding the root growth and water uptake in deep soil layers and the potential limitations associated to such conditions. As intermediate wheatgrass is a monocotyledonous crop and alfalfa a dicotyledonous crop, this study also investigates the differences between the two crop types in terms of root anatomy, growth and water uptake.

Crop water uptake was monitored by combining the use of soil moisture sensors and water stable isotope techniques. In parallel, crop root growth was monitored using minirhizotron tubes and soil core extraction. An underlying goal was to compare the different scientific approaches and identify their advantages and dis-advantages under field condition. A water flow model along the plant-soil-atmosphere continuum (HYDRUS-1D) was used to obtain quantitative estimates of root water uptake from empirical data. The particularly dry weather during the 2018 season allowed the study of these two perennial crop species under very different water conditions, which brought interesting perspectives in terms of water use under drought. At the plant level, in depth characterisation of root anatomy was realised to understand the potential anatomical restrictions to water flow within the root system. This study focused on identifying anatomical differences associated to increasing soil depth and between the two crops. Particular emphasis was put on understanding the physical or physiological limitations to root growth and water uptake under field conditions.

This PhD project was funded by the Villum Foundation (DeepFrontier project, grant number VKR023338).

Deep Frontier is a research collaboration between University of Copenhagen, Aarhus University and International Centre for Research in Organic Food Systems (ICROFS), financed by the Villum Foundation.

Preface

This thesis manuscript consists of five chapters. The first chapter constitutes an in-depth literature review on major physiological and anatomical processes affecting root growth and water uptake at the plant level. The second chapter aims at replacing crop growth and water uptake in broader agricultural context. These two chapters, which constitute the literature review of this thesis, are not intended to be exhaustive but rather provide a good overview of the temporal and spatial variability of the abiotic and biotic factors that influence plant performances in the field. Special focus was put on processes affecting root growth and functioning at great soil depth. The overall goal was to construct a strong background to support the PhD work and to explain how, when and why root systems could be approached as tools for agricultural management. Wherever possible, examples on wheat, alfalfa and intermediate wheatgrass were used because of their relevance to the study and the amount of information available. The third and fourth chapters constitute the two scientific papers to be published alongside this thesis. They focus on perennial crop root anatomy and water uptake at great soil depth, respectively. The fifth chapter is a general discussion of the work that summarizes the main findings and puts the work into perspective. I am the first author of all the content found in this manuscript and co-authors' contribution to these two manuscripts is detailed at the bottom of this thesis. Note that the numbering of figures and tables was set back to one at the beginning of the third and fourth chapters.

Table of contents

I. State-of-the-art

Deep roots a broad understanding

Defining deep roots

In the literature, the term deep roots can be misleading as all roots in “relatively deep soil layers” are called “deep roots”. Because maximum sampling depth is often shorter than maximum rooting depth, defining deep roots is relatively limited by the lack of measurements. A study by Schenk and Jackson (2002) estimated the median sampling depth at 0.88 m, over 475 root profiles. More recently, Fan et al. (2016b), attempted to quantify the root distribution of common agricultural crops and the average sampling depth over the 81 studies included was found to be 0.94 m. Based on this observation, Maeght et al. (2013) proposed a 1-m threshold and defined deep roots as roots growing below 1 m soil depth. Although this method constitutes an oversimplification, it remains relatively simple and practical as discussed by Pierret et al. (2016). In natural habitats, trees and shrubs are considered to grow deep roots with, on average, a reported maximum rooting depth of 7.0±1.2 m and 5.1±0.8 m, respectively against 2.6±0.1 m for herbaceous species (Canadell et al. 1996). However, rooting depth vary substantially across terrestrial biomes mostly driven by differences in climates, species and soil conditions (Fig. 2).

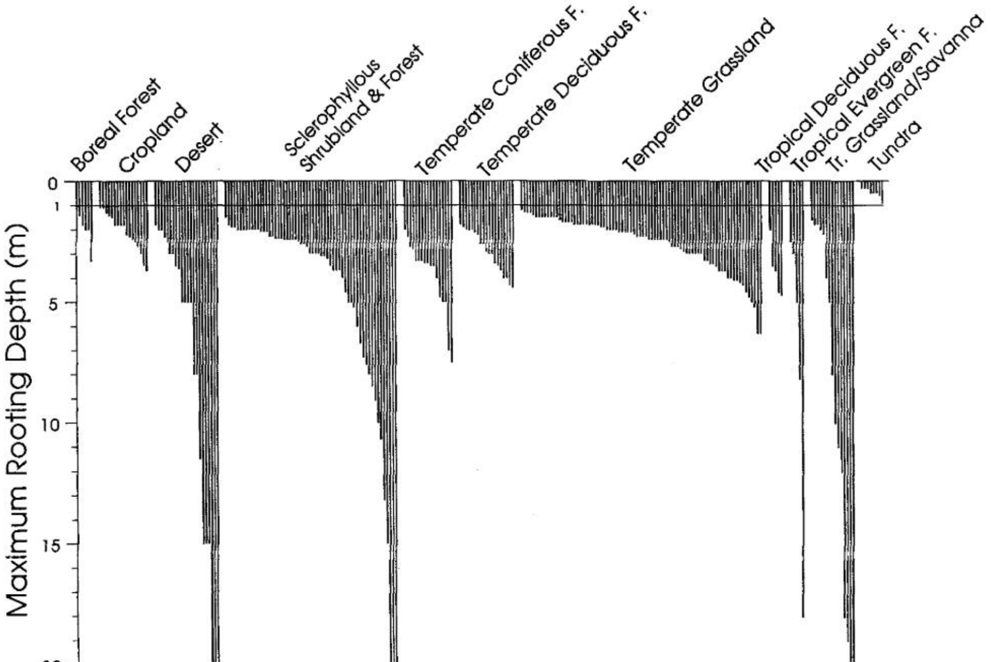


Figure 2. Deepest maximum rooting depth reported for 253 species growing across 11 terrestrial biomes (from (Canadell et al. 1996))

Deep rooted crops

When focusing on agricultural crops, a review by Borg and Grimes (1986) of 48 crop species from 134 field observations under favourable environmental conditions, concluded that for many crop species the maximum rooting depth ranges between 1.5 to 3.0 m with deepest rooting depth found for sugarcane and alfalfa at 6.0 m. However, an important factor to consider is that they considered solely crops grown under favourable environmental conditions. While their findings certainly highlight the physiological potential of crop root system, such potential might be restricted at other locations due to constraints to root growth (e.g. soil, climate). Fan et al. (2016b) did a fair attempt to characterise the root distribution of common temperate crops and concluded that, for most crops, roots lie between 0.5 and 1.5 m (Fig. 3a). They also found that alfalfa was the deepest rooted crop and that roots of most cereals decrease more steeply with depth.

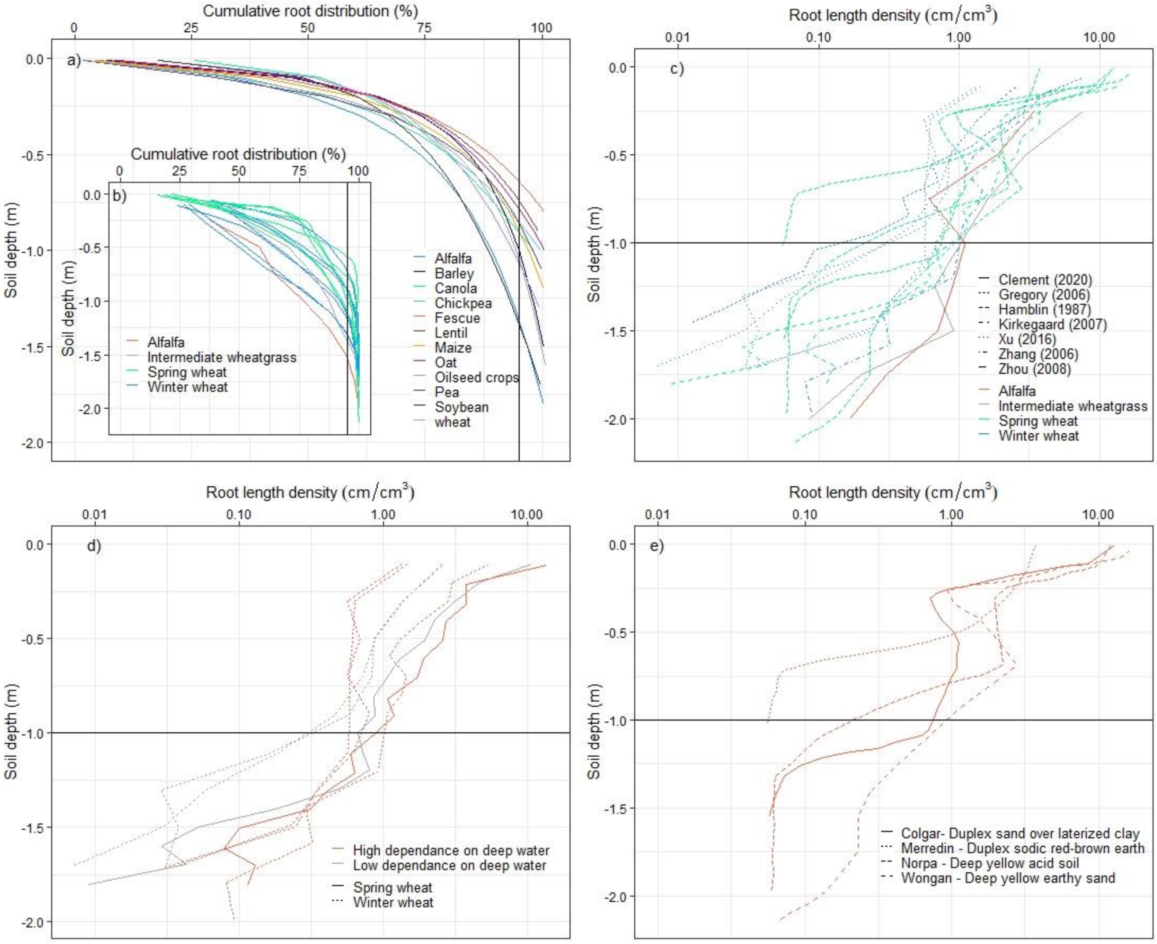


Figure 3. (a) Cumulative root distribution of common temperate crops estimated by (Fan et al. 2016b); (b) Cumulative root distribution and (c) root length density evolution with depth of alfalfa, intermediate wheatgrass, spring and winter wheat found in field studies that investigated at least 1.8 m soil depth. Extracted from (Hamblin and Tennant 1987; Gregory 2006; Zhang et al. 2006; Kirkegaard et al. 2007; Zhou et al. 2008; Xu et al. 2016); (d) Root length density evolution with soil depth of spring and winter wheat reported in study that studied the effect of subsoil water availability (i.e. below 1 m soil depth) on crop performance and root growth when topsoil layers dries up (Zhang et al. 2006; Kirkegaard et al. 2007; Xu et al. 2016). Drying of the topsoil layer was triggered either by drought inducement or low irrigation treatments; (e) Root length density evolution with soil depth reported for spring wheat at four different locations by (Hamblin and Tennant 1987). Data were extracted using WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/)

However, when focusing on field studies on winter and spring wheat which considered a "deeper" sampling depth of at least 1.8 m, it was found that the shape of the exponential decrease in root distribution with depth is quite variable (Fig. 3b). While some profiles are similar in shape with the one reported by Fan et al. (2016b), others diverge, probably because, when the sampling depth is shallow, shallow roots outweigh deep roots when expressed in relative proportion as discussed by Pierret et al. (2016a). Overall, winter wheat grow more roots at depth while spring wheat has more roots in the upper soil layer and a more pronounced decrease in root distribution with depth. When including results from this thesis, alfalfa was found to be the deepest rooted crop while the root distribution of intermediate wheatgrass was similar to winter wheat. However, actual root length density per se at depth was found highly variable across crop types (Fig. 3c) and suggest important influence of the crop cultivars, site conditions and crop management. Studies from (Zhang et al. 2006; Kirkegaard et al. 2007; Xu et al. 2016) investigated the effect of subsoil water availability (i.e. below 1 m soil depth) on crop performance and root growth when topsoil layers dries up (Fig. 3d). Interestingly, results from these studies converged and wheat grown at sites with higher dependence on deep soil water tend to present higher root length density at depth highlighting the plasticity of the root system. A study from Hamblin and Tennant (1987) further supports this idea of root growth plasticity as root length density profiles of similar spring wheat cultivars grown at four locations in Australia were found quite variable, especially due to differences in soil type (Fig. 3e). In conclusion, a large range of crops can develop roots below common investigation depth (i.e. 1 m) but deep rooting is probably a trait that is as common as variable. In agricultural systems, variability in crop rooting depth are most likely to originate from differences in (1) plant species and genotypes (G), (2) environmental conditions (E) and (3) crop management (M) (Thorup-Kristensen and Kirkegaard 2016). Understanding these GxExM interactions is a pre-requisite for achieving deep rooting and understanding their potential for deep water uptake.

Root growth and root system morphology

Root growth rate and seasonal development

Roots elongate longitudinally by a combination of cell division and elongation that occurs close to the root tip. A growing root possess four regions of cell elongation (Fig. 8a), (1) the root cap that is pushed forward and is involved in cell division and in sensing the environment, (2) the meristematic region where cell division and differentiation occurs, (3) the elongation region where cells elongate and the (4) maturation region where old root tissues evolve over time (see section "The composite structure of root") (Hodge et al. 2009). Root growth, over the plant growing cycle, usually follows a logistic curve with high root growth during the vegetative stage which later decreases toward the reproductive stage (Fig. 4a). Assimilate allocation to root growth change from approximately 50% to 5% from the vegetative to the reproductive stage (Anderson-Taylor and Marshall 1983). Crops show wider range of root depth penetration rate depending on the species, with rates ranging between 0.2 and 3.0 mm °C day^{-1} for leek and winter rape, respectively (Thorup-Kristensen 2001; Kristensen and Thorup-Kristensen 2004). Taking wheat as an example, root depth penetration rates have been reported ranging between 1.2 to 1.8 mm °C day^{-1} (Barraclough and Leigh 1984; Kirkegaard et al. 2007; Thorup-Kristensen et al. 2009) but values higher than 1.5 mm °C day^{-1} could be found under unconstrained conditions. Rooting depth usually increase linearly with accumulated temperature until anthesis where root penetration ceases (Thorup-Kristensen et al. 2009). Therefore, the time of sowing influences the crop rooting depth. Early sown crops usually develop a deeper and more extensive root system due to their longer vegetative phase and higher temperature accumulation before anthesis (Barraclough and Leigh 1984). Similarly, winter crops due to their longer growing period are known to be deeper rooted than summer crops (Fig. 4b). For example, winter wheat was found to reach on average 2.2 m against 1.1. m for spring wheat, at the end of the season (Thorup-Kristensen et al. 2009). Perennial crops, with longer growing period are often found to be deeper rooted than annual crops, see section "Annual vs perennial crops".

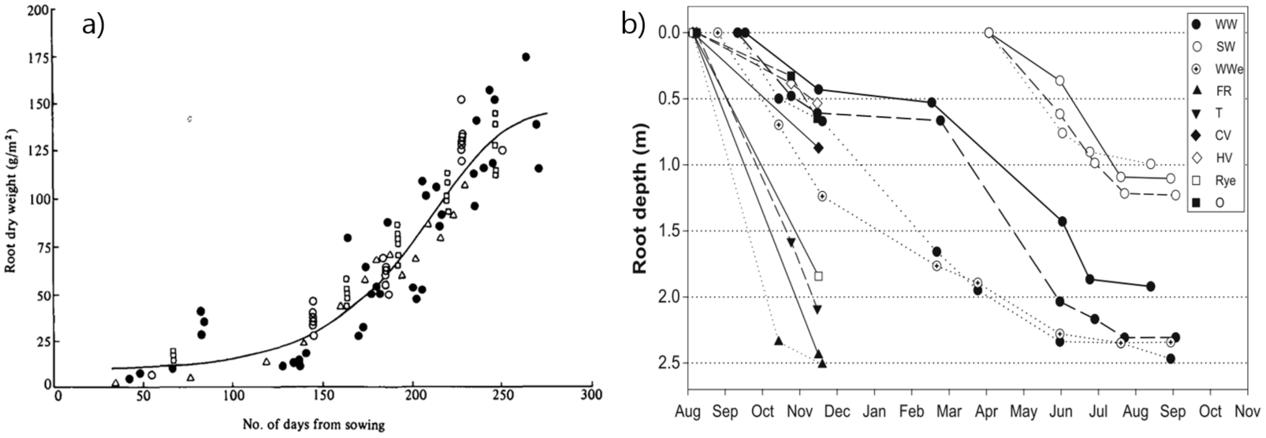


Figure 4. (a) Root dry weight evolution in relation to day of sowing of winter wheat. Compilation of various studies (point-shapes) from (Barraclough and Leigh 1984). (b) Evolution of the rooting depth over time of different cereals and catch crops. Linetype differences refer to different years. WW: Winter Wheat; SW: Spring Wheat; WWe: early sown winter wheat; FR: Fodder Radish; T: Turnip; CV: Common vetch; HV: Hairy Vetch; O: Oats from (Thorup-Kristensen et al. 2009)

Root system architecture

When focusing belowground, the plant accessibility of soil resources depends largely on the root system architecture i.e. the development and spatial arrangement of the root system within the soil. Root system architecture is complex and requires understanding of root development, topology and of the interconnection between the growing environment and the plant response (Lynch 1995). The root system architecture is thus a dynamic notion that is very flexible and variable in time, space and across species (Fig. 5).

When applying a simplified view, monocotyledonous and dicotyledonous species have very different root system architecture. Dicotyledonous species like alfalfa, generally display a taproot from which different levels of lateral roots derive (Fig. 6bc). In monocotyledonous species such as intermediate wheatgrass, seminal roots emerge first from the seeds whereas nodal roots emerge later on from the shoots (i.e. tillers, stems) (Fig. 6a). Lateral roots emerge from both root types from lateral root primordia located in the pericycle, see section "The composite structure of root". There is genetic variability in root system architecture, for example wheat and maize cultivars are known to differ in their number of seminal and nodal roots (Richards and Passioura 1981a, b; Gao and Lynch 2016). Variability in alfalfa branching density are also observed (McIntosh and Miller 1980). Lateral root density at the root system scale is highly variable and depends on crop genetic variability and their response to environmental conditions (Muller et al. 2019). However, very little is known about this variability in time and space under field condition. Variability in root architectural phenotypes could be beneficial in agriculture to address challenges such as drought or nutrient deficiency, see review from Koevoets et al. (2016) and section "Timing, efficiency and partitioning of soil resource use").

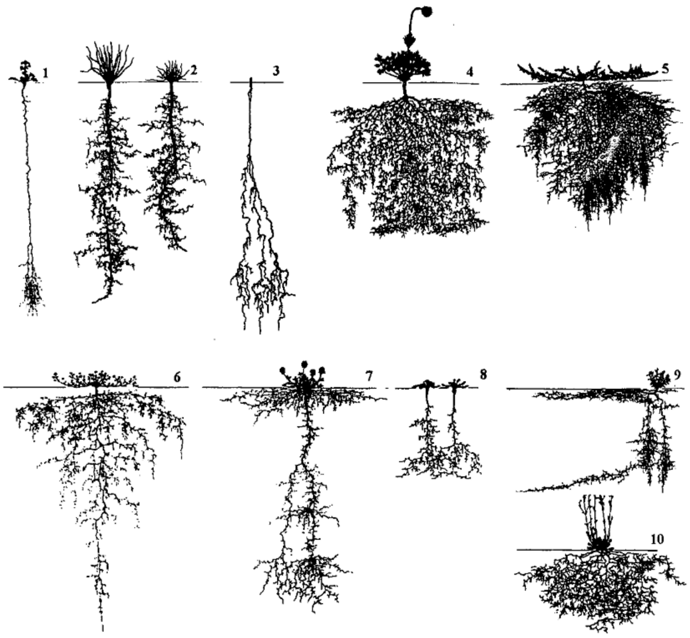


Figure 5. Root system architecture of some dicotyledonous plants. 1- Fryngium campestre; 2- Scorzonera. villosa; 3- Chondrilla juncea; 4- Pulsatilla pratensis; 5- Genista germanica; 6 -Trigonella balansae; 7-Trifolium trichocephalum; 8- Carum caucasicum; 9- Onosma arenarium; 1O- Silene otitis. Drawings from (Kutschera and Lichtenegger 1992) taken from (Lynch 1995)

Root type and functions

Individual root type is usually determined from the root origin in the root system (i.e. ontogenesis) (Fig. 6). Differences in time and location of emergence, morphology and anatomy are common across root types and lead to differences in their role and functions, particularly regarding the supply of water and nutrients. For the purpose of this study, particular emphasis was placed on functional differences between root types in water uptake but differences in nutrient uptake also exist. In monocotyledonous plants, the seminal root system mainly provides water during early stage of development whereas the nodal root system constitutes the principal source of water supply for mature plants. For example, seminal roots of 17-days-old barley plants were found to

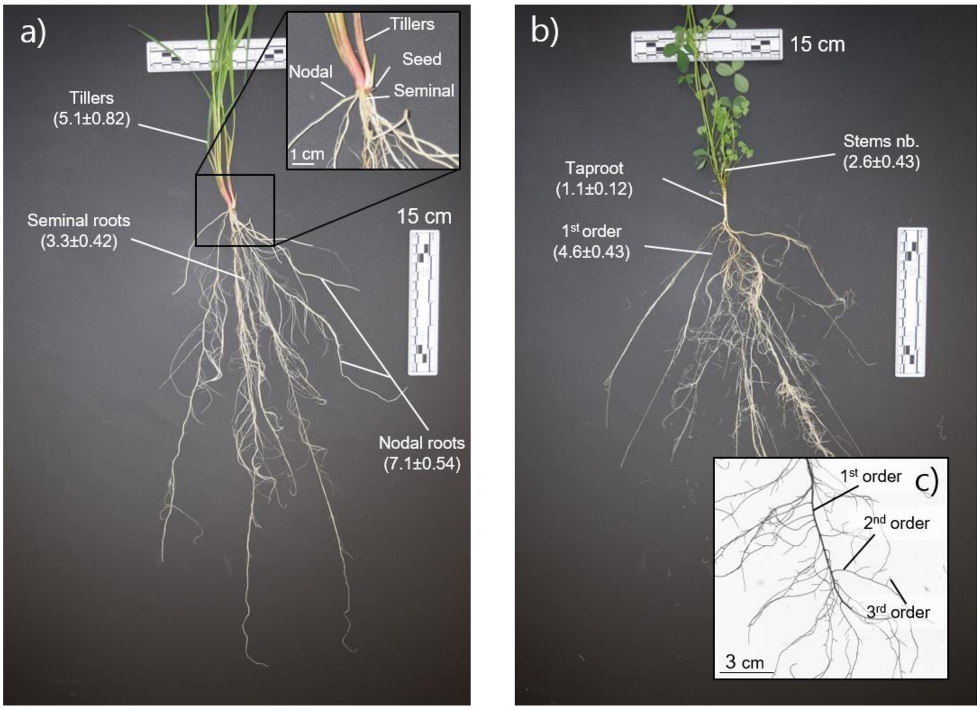


Figure 6. Root system architecture of intermediate wheatgrass (a) and alfalfa (b) of 47-d old plants, at approximately, the node formation and early vegetative stage, respectively. (c) Scanned 1st order lateral root of alfalfa. Nodal, seminal, taproot and four different order of lateral roots are indicated with average ± standard error over eight plants (From Clément et al., Forthcoming)

contribute up to 92% of the plant water uptake (Knipfer and Fricke 2011). The lower contribution of nodal roots were due to lower number of roots, lower surface area and lower axial hydraulic conductivity when compared to seminal roots. However, this pattern is expected to change over time. Indeed, a study from Ahmed et al. (2018b) on 5-weeks-old maize plants concluded that most plant water uptake was done by the nodal root system. They identified that this functional difference was driven by increased axial hydraulic conductance, fewer and shorter lateral roots and to nodal roots being connected higher up along the shoots in comparison to seminal roots. In addition, the same authors showed that water uptake occurs preferentially in lateral roots while parent/axial roots are preferentially used to axially transport water to the shoots of young maize plants (Ahmed et al. 2016b). In dicotyledonous species, root type is usually determined from the root order within the root system with the lowest order root (i.e. 1^{st}) commonly being the taproot (Fig. 6b). In general, functional differences that occur between root types are the result of differences in aging and tissue differentiation, in particular, differences in secondary growth. Vercambre et al. (2002) found that fine roots of Prunus had a lower axial hydraulic conductivity associated to lower increase in xylem vessels number during secondary growth. In contrast, they found that lower order "woody" roots presented a higher axial hydraulic conductivity due to important increase in xylem vessels during secondary growth. As summarized in the review of Mccormack et al. (2015), for root of dicotyledonous species, uptake capacity, root respiration and mycorrhizal colonization usually increase with root order, while secondary thickening, transport capacity, lifespan and C:N ratio tend to decrease (Fig. 7). Functional differences between root types are of great importance as they influence the use of soil resources. In the case of water, functional differences associated to root types are mostly driven from differences in anatomy and maturation processes that will be described in the section "The composite structure of root".

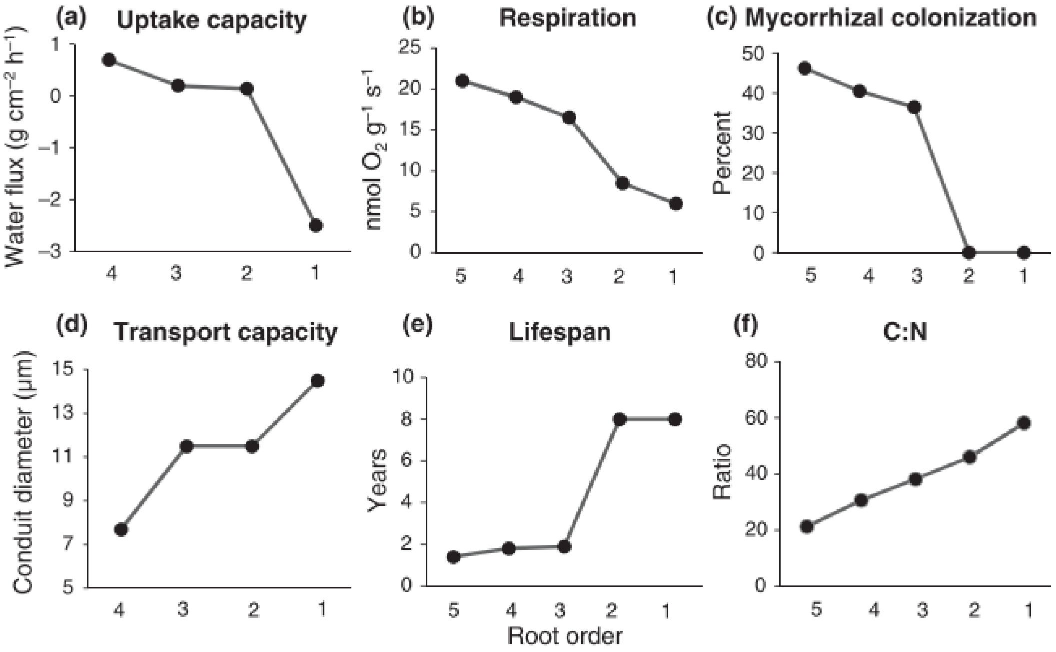


Figure 7. Functional differences associated with root order of dicotyledonous species from (McCormack et al. 2015)***. A root order of 1 refers to the taproot***

Root growth response to soil conditions

Root growth response to heterogeneous soil resources

Root growth, density and trajectory in the soil are driven genotypically, however different tropisms apply. Three major tropisms occur in the soil which are (1) gravitropism, the plant response to gravity, (2) chemotropism, the plant response to different level of nutrient concentration and (3) hydrotropism, the plant response to different level of soil water content. Gravitropism plays an important role on root growth by influencing the growth angle with which roots penetrate the soil. For example, rice cultivars with steeper growth angle were found to be deeper rooted and less prone to drought (Uga et al. 2013). In contrast, common bean cultivars with a broader growth angle presented a shallower root system and a greater phosphorus acquisition as phosphorus predominates in the topsoil (Ge et al. 2000). Ultimately the root angle partly determines the zone and depth of soil exploitation which is of great importance when trying to optimize soil resource use. Chemotropism can be seen as a trait that aims at optimizing the exploitation of soil nutrients that are naturally dispersed in patches of different concentrations. A common plant response to such patchy distribution of soil resources is usually increased root growth or nutrient uptake in nutrient-rich patches, for a review see Hodge (2006). For example, compensatory root growth, via increased root branching, were identified in a portion of a root system of barley exposed to increased nitrogen and phosphorus availability (Drew and Saker 1975, 1978). Similarly, increase in root growth at depth were observed for Chinese cabbage, summer squash and white cabbage as a response to deep nitrogen availability (Kristensen and Thorup-Kristensen 2007). However, plants are expected to differ in their response. Hydrotropism also influences root growth as roots tend to bend toward areas of higher water content, and a recent study allow more precise understanding of the mechanisms behind such phenomenon (Dietrich et al. 2017). However the magnitude of this response in the field is still unclear. Another form of hydrotropism to consider is the increased root growth in deep soil layers as a response to topsoil drying under drought, as identified for chicory (Vandoorne et al. 2012) and spring wheat (Kirkegaard et al. 2007) (Fig. 3d). Naturally, water availability and distribution in the soil can have important influence on root growth, see section "Soil water content influence on root growth". In the end, chemotropism and hydrotropism could occur at the root and root system level, the latter having necessarily a greater influence on the whole plant functioning.

Soil physical constraints to root growth

Plants can cope with heterogeneous soil conditions *via* their physiological and morphological plasticity. Root growth can be constrained by a large number of soil physical and chemical properties that are highly variable in time and space. Constraints to root growth usually increase with depth and deep roots by nature grow in subsoil horizons (i.e. B, C or E) that present different characteristics compared to topsoil horizons (i.e. O and A). Root growth is usually constrained by (1) soil mechanical strength, (2) aeration and (3) temperature. Soil mechanical strength is one of the major physical limitations to root growth in agricultural fields (Valentine et al. 2012). Soil mechanical strength usually increases with soil depth and soil drying, irrespective of the soil management (Gao et al. 2016). Capacity of roots to grow in strong soil layers is species and cultivar dependant and steep, stiff and thick roots are usually associated with improved penetration of compact soil layers, for a review see Jin et al. (2013). In particular, it was shown that wheat cultivars with improved penetration capacity where deeper rooted under field conditions (Botwright Acuña et al. 2007). Root growth is more sensitive to axial resistance than radial resistances (Bengough 2012), explaining why roots grow preferentially in macropores (e.g. old root channels, earthworm channels, cracks), particularly in subsoil layers (White and Kirkegaard 2010). Thus, the capacity of roots to locate subsoil pore structures is suggested to be an important trait for deep rooting (Colombi et al. 2017). However, poor root-soil contact or clumping of roots in the same pores could limit resource exploitation in those pores and counterbalance the advantage of preferential root growth (Passioura 1991; Stirzaker and Passioura 1996).

Nonetheless, plants develop mechanisms to cope with air gaps that could form at the root-soil interface such as root hairs and mucilage (see section "Maintaining hydraulic continuity at the root-soil interface").

Oxygen deprivation often restricts root growth and functions as oxygen diffusion from the soil surface into the soil profile is mostly diffusive. Thus, hypoxic or anoxic conditions (i.e. partial or complete lack of oxygen), usually increase with soil depth due to soil structure or water saturation (i.e. macropores are filled due to rising of groundwater). Root growth and functions under anaerobic conditions depends on the tolerance level and avoidance mechanisms used by the plant (reviewed by Vartapetian and Jackson (2015)). In particular, plants which can increase oxygen transport through aerenchyma formation or reduce their respirational needs *via* reduced cortical area were found beneficial in maize and barley (Jaramillo et al. 2013; Schneider et al. 2017). Such advantageous traits are supposed to confer enhanced root elongation under low oxygen conditions and enhance deeper rooting as suggested by Lynch and Wojciechowski (2015) but empirical evidence are lacking. Plants that developed strong barriers to oxygen leakage, most likely through suberin deposition, are also supposed to grow deeper roots under saturated conditions, due to maintenance of adequate apex oxygenation (Striker 2012).

Low soil temperature, is commonly known to reduce root growth of common crops like spring barley, oilseed rape and maize (Macduff et al. 1986; Nagel et al. 2009). Ueda (1936) noticed that wheat cultivars with greater cold resistance had deeper roots in early growth stages. In the field, soil temperature follows a characteristic vertical gradient with soil depth associated with high diurnal and seasonal fluctuations in upper soil layers, but more constant, temperature in the subsoil (Walter et al. 2009). Temperature in the subsoil are usually lower in spring and early summer due to the delayed warming of deep soil layers compared to topsoil layers. In temperate regions, subsoil warming increases downwardly throughout the season and can limit the plant rooting depth (Kaspar and Bland 1992). Tolerance to low temperature is genotype dependent and breeding for low temperature tolerance may increase crop rooting depth (Hund et al. 2007).

Soil chemical constraints to root growth

There are two major soil chemical properties that can limit root growth (1) nutrient deficiency and (2) toxic levels of chemical elements. Plants require 17 nutrients for growth and development. Macronutrients (i.e. C, H, O, N, P, K, Ca and Mg) are required in large quantity whereas micronutrients (i.e. Zn, Cu, Mn, Fe, B, Mo, Cl and Ni) are required in minor quantity. However, deficiency in any of these nutrients will induce reduction of plant growth, usually in the order of P > Ca > Mg > N = K > S for macronutrients and B > Zn > Cu > Fe > Mn > Mo for micronutrients for dry bean (Fageria and Moreira 2011). In agricultural systems, N and P constitute the most important nutritional limitation worldwide and increasing efficiency for these two nutrients is considered a top priority (Lynch 2019). Nitrogen, in the form of nitrate, is mobile and can be rapidly leached down the soil profile, while phosphorus is immobile and will predominately stay in the topsoil. These spatial differences in resource pools along the soil profile could affect the plant nutritional status and require particular attention in crop management, see section "G x E x M"). Ultimately, shoot and root growth are intrinsically linked to the nutrient distribution and availability in the soil. As mentioned earlier, crops show different morphological and physiological responses to the presence of local nutrient deficiency and nutrient-rich patches in the soil (Lyu et al. 2016). However, field applications of such plant characteristics are often limited by the level of understanding of root characteristics and their response to a heterogeneous and changing environment (Garnett et al. 2009). Soil acidity ($pH<5.5$) or alkalinity ($pH >8.5$) is common in humid and arid environments and considerably limits agricultural production worldwide. 40 to 50% of the world’s arable lands are acidic with approximately 60% of acid soils being located in the tropics. In acidic soil, the major constraints to root growth are toxicity of aluminium and manganese associated to deficiency in phosphorus, calcium or magnesium (Baligar et al. 1998). Plants can develop tolerance mechanisms (i.e. exclusion, detoxification/sequestration) to aluminium toxicity for example and breeding programs are trying to target such traits (Kochian et al. 2015). However, another interesting approach could be to look for efficient capture of soil nutrients under acidic soil conditions, which has received much less attention (Lynch 2019). Acidity in the subsoil layers is frequent and constitute a growth limiting factor for agricultural crops. Advances in crop modelling, particularly in comparing information from yield maps, could offer interesting prospects to target areas with acid subsoil and to accordingly adapt the cropping management (i.e. deep vs. shallow rooted crops) (Wong et al. 2008). Alkaline soils are also relatively frequent and usually associated with high amount of calcium carbonate ($CaCO_3$). Increase of the soil pH decrease the availability of Fe, Zn, P, Mn and B, which further constrains root and shoot growth. Large differences occur in Fe, Zn and Mn efficiency among crop species and cultivars and offers good potential for crop selection and breeding programs under alkaline soil conditions. In particular, crops that could release proton via their roots and therefore acidify the rhizosphere showed higher uptake of nutrients (Zhou et al. 2009). However, physiological mechanisms are still not fully understood, for a review see George et al. (2012). In addition, herbaceous plants can also develop adaptation mechanisms such as the production of calcified roots (i.e. deposition of $CaCO_3$ in their cortical cells) under alkaline conditions.

Soil salinity is also an important limiting factor for crop production worldwide as it reduces root growth by creating osmotic stress to the roots and reduces water uptake. High concentration of soluble salts in the soil usually originates from intensive irrigation, evaporation and saline groundwater rising, especially in arid and semi-arid regions. Plants developed three distinct response mechanisms (1) tolerance to osmotic stress, (2) salt exclusion and (3) tolerance to accumulated salt in plant tissues. However, plants differ greatly in their tolerance level. For example alfalfa was found to be very tolerant while cereals like rice and wheat were found to be the most sensitive (Munns and Tester 2008). Genetic differences in salt tolerance exist among crop cultivars and within species which also offers good prospect for breeding and improvement of agricultural production in saline-prone environments.

Soil water content influence on root growth

As previously mentioned, the soil water content indirectly influence root growth by inducing oxygen deficiencies under saturation or increasing soil mechanical strength under drying. Therefore, the Least Limiting Water Range (LLWR) parameter was developed to describe the soil water content range within which roots can grow without being restricted by soil mechanical impedance or aeration (da Silva and Kay 1997). This indicator when coupled to soil water content evolution could be used to track seasonal physical stresses that apply to root growth. However, a relatively high amount of information is required, limiting field application. A simpler parameter was determined by Dexter (2004), the so called S-parameter that corresponds to the slope of the soil water retention curve at its inflection point and is used as an indicator of soil physical quality and overall describe the rootability of a soil layer. Obviously, when no other physical restriction occurs, roots grow preferentially in soil layers with optimal soil moisture content (i.e. ~field capacity). When soil moisture content decreases in a specific soil layer, root growth is usually reduced in that layer but does not necessarily stop (Sharp and Davies 1985). Root growth is reduced due to higher mechanical strength and low water availability. Naturally, soil drying has also a direct effect on root growth by limiting the plant water availability due to decreased soil water potential or/and reduced soil/root contact due to root and soil shrinking. Plants have different mechanisms to respond to heterogeneous soil water distribution but the magnitude of the response depends on the water distribution, soil physical characteristics and on the severity of the drying of the soil profile. In a context where only some of the root zone is dry, root growth can be redirected to the remaining "wet" zone, which is usually deeper in the soil profile. This is common in regions with in-season rainfall variability but stored water at depth. For example, maize plants exposed to topsoil drying were found to reduce their root growth in the upper soil layer but increase root proliferation in deep soil layers (i.e. >60 cm) in a pot experiment (Sharp and Davies 1985). Similarly, increase in root length in the bottom part of pots (i.e. >60 cm) exposed to topsoil drought were observed for maize but not for rice (Kondo et al. 2000). This latter information is important as compensatory root growth is not always occurring. A field study by Kirkegaard et al. (2007) showed significant increase in root length density between the 1.0-1.2 m soil layer for wheat exposed to topsoil drying but with access to deep water. In situations where soil drying affect most of the rooting zone, no compensation will occur and overall root growth will be reduced. This is common in water-limited environments with limited stored water. Here focus is made on physical parameters limiting root growth under soil drying but soil chemical parameters are also important as nutrient availability decrease under drying. The best illustration of this comes from a study from Barraclough et al. (1989) who studied the influence of topsoil drying and nitrogen availability on root growth in a field with a relatively compacted subsoil. They observed that compensatory root growth occurred under drought when high level of nitrogen was applied to the field but not under the low nitrogen scenario. They also conclude that such increase in root growth occurred only down to 80 cm and that below that, the compacted soil structure was probably limiting deep root growth. This study highlights perfectly the interconnection between physical and chemical constraints to root growth that occur in sequence or combination in the field, see review from Whitmore and Whalley (2009). The magnitude of these constraints on root growth depends on many parameters, such as soil type, parent material, climate, soil pH, chemical composition, farming practices and type of crops and cultivar being grown. Unfortunately prediction of these constraints and of the plant response are difficult due to temporal and spatial variability and the lack of empirical data. In addition, plant physiological and morphological responses to soil heterogeneity are still relatively unknown due to the lack of consideration of field like soil-plant system in experimental settings.

The composite structure of root

Root structure is complex due to the wide diversity of cell types and maturation processes that differ with root type, age and plant species. Due to the lack of mature xylem vessels the root cap, meristematic and elongation regions are hydraulically isolated (Fig. 8a). Therefore, this chapter describes a theoretical mature root, which comprise most anatomical structures encountered in young roots of both dicot- and monocotyledonous plants. When looking at a root radial cross-section (Fig. 8b), one can see layers of cells arranged in series, from outside to inside: Outside < Root hair/epidermis < exodermis < cortical cells < endodermis < pericycle < parenchyma < tracheary elements (i.e. xylem & phloem) < inside. Overall, they form two major tissues (1) the cortex, from root hair to the endodermis and (2) the stele, from the pericycle to the inner center where tracheary elements are disposed. This section presents the main characteristics of these different cell layers with a special focus on their relation to plant water transport. Therefore, phloem was considered outside the scope of this review, due to its primary role in the transport of mineral elements however its role in plant signaling should not be forgotten.

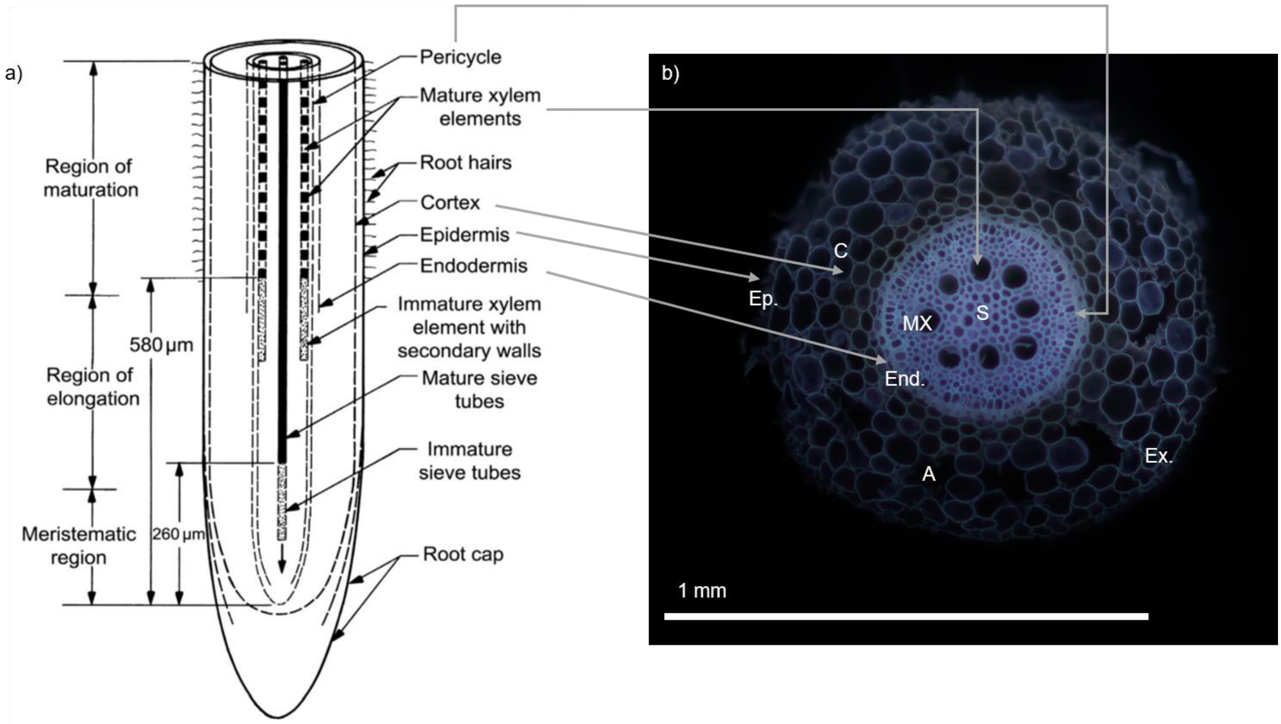


Figure 8. The composite structure of roots. (a) Root structure and elongating regions. The distances vary with crop species, root types and growth rate (Kramer 1983); (b) Root cross section of intermediate wheatgrass nodal root ablated at 20 cm from the soil surface at 107 DAP grown on a sandy loam. Annotations refers respectively to (A) Aerenchyma, Epidermis (Ep.), Exodermis (Ex.), Endodermis (End.), MetaXylem vessel (MX), Cortex area (C), Stele area (S) (from Clément et al, XX). Note: location of the exodermis is hypothetical here as presence and suberization of the exodermis is not evident in this image

The root epidermis and root hairs

The epidermis, the outermost cell layer that surrounds plant tissue constitutes a boundary between the plant and the environment. Its aim is to fulfill two contrasting roles which are to (1) protect the plant tissues against biotic and abiotic factors and (2) to control and permit the exchange of gas, water and nutrients with the environment (Javelle et al. 2011). Two types of cells constitute the root epidermis, trichoblast cells which can form root hairs and atrichoblast cells which cannot. Plant species present different patterns of trichoblast/atrichoblast presence in the epidermis (Marzec et al. 2015). Root hairs can vary in length and density but confer the advantage of increasing the root surface area and thus the volume of soil exploited while minimizing the metabolic cost. A number of studies have now highlighted the key role they play in plant water and nutrient uptake, especially immobile nutrients (York et al. 2013) but they also provide other functions such as anchoring (Tester and Leigh 2001). Root hair density and length are highly regulated by environmental conditions especially nutrient concentration, with increasing root hair under scarcer nutrient availability (Grierson et al. 2014), however evidence in the field are lacking. Previous studies showed that soil porosity, particle size and water content also impacted root hair length with shorter root hair under wet conditions and in soil with high mechanical strength (Hammac et al. 2011; Haling et al. 2014). From the author's observation on roots growing in mesocosms and rhizoboxes, the lack of root-soil contact appears to be an important trigger in the formation and length of root hairs. For more details on their role and functions see section "Maintaining hydraulic continuity at the root-soil interface".

The root cortex

The cortex is the part of the root between the epidermis and the stele (Fig. 8b). It consists of multiple files of unspecialised cells, formed after several cell divisions that happen at the root apical meristem (Lux et al. 2004). The cortex can be divided into three specific areas, the exodermis (i.e. a single cell file located right below the epidermis), the endodermis (i.e. a single cell file located in the inner part of the root cortex) and the mesodermis (i.e. all cell files located in between the endodermis and the exodermis (Fig. 8b). Cortical cell file number and cell size are quite variable across root type and order (Lux et al. 2004), plant species and cultivars (Bramley et al. 2009; Chimungu et al. 2014a). Inner and outer cortical cell files are usually smaller in size and are assumed to be related to their respective roles of regulating the root radial transport and protecting the root against pathogens, see section "The root water pathway". Whereas mesodermal cells are more involved in aeration and oxygenation of the root via aerenchyma formation and storage of reserve material (i.e. starch). As proposed by Lux et al. (2004), the entire cortex could be seen as a "buffer zone" or a "temporary tissue" and in many case will disappear due to ontogenesis or deterioration, see section "Root structure evolution and maturation".

The endodermis and exodermis: Two specialized cortical cell layers

In the inner and outer part of the root cortex, there are two specialized unicellular cell layers called the endodermis and exodermis, respectively. Note, that while most vascular plant develop an endodermis, the presence of an exodermis is less frequent and does not always constitute a uniform sheath around the root. These cell layers can develop specific cell wall modifications that are classified into three categories depending on their development and maturation stages, (1) Stage I: deposition of lignin in the cell wall forming casparian bands, (2) Stage II, presence of suberin lamellae in the inner cell wall and (3) Stage III, a tertiary wall is formed internal to the suberin lamellae due to deposition of lignified cellulose (Fig. 9) (Sanderson 1983; Lersten 1997; Lux et al. 2004; Schreiber and Franke 2011). While the three stages of cell wall development can be observed for endodermal cells only the two first have been described in the case of exodermal cells (Fig. 9). However, maturation of cells to stage II and III are not a synchronous process and some endo- and exodermal cells will mature later on, or remain in stage I, depending on the plant species. Those cells, which lack suberin lamellae, are called passage cells. In the endodermis, passage cells usually align with protoxylem vessels whereas they follow a more patchy pattern in the exodermis (Peterson and Enstone 1996). Due to the hydrophobic characteristics of casparian bands and suberin lamellae, the endodermis and exodermis are now widely recognised as an effective barrier to mass-flow of water and nutrients into the stele area (Hose et al. 2001). Hence, passage cells, which are delayed in their maturation process, constitute a lower resistance pathway to solutes and water. In most plants, an endodermal casparian band develops within 10 mm from the root tip and prevents uncontrolled flow whereas exodermal casparian band develops within several centimeter away from the root tip and isolate the root from hostile environmental conditions (i.e. pathogens, salt, drought, anoxia) (Enstone et al. 2003; Geldner 2013). The exodermis, due to its position at the root surface allow roots to be selective in term of water and solute uptake, prevent pathogens and toxic compounds from reaching the root and prevent oxygen loss under oxygen deficiency (Reissinger et al. 2003; Chen et al. 2011; Watanabe et al. 2013). Overall, these two physiological sheaths are key traits that allow plants to cope with a constantly evolving environment and are considered to be key traits for abiotic stress tolerance especially salinity, drought and anoxia and therefore are advantageous for root growth and activity in deep soil layers (Tylová et al. 2017).

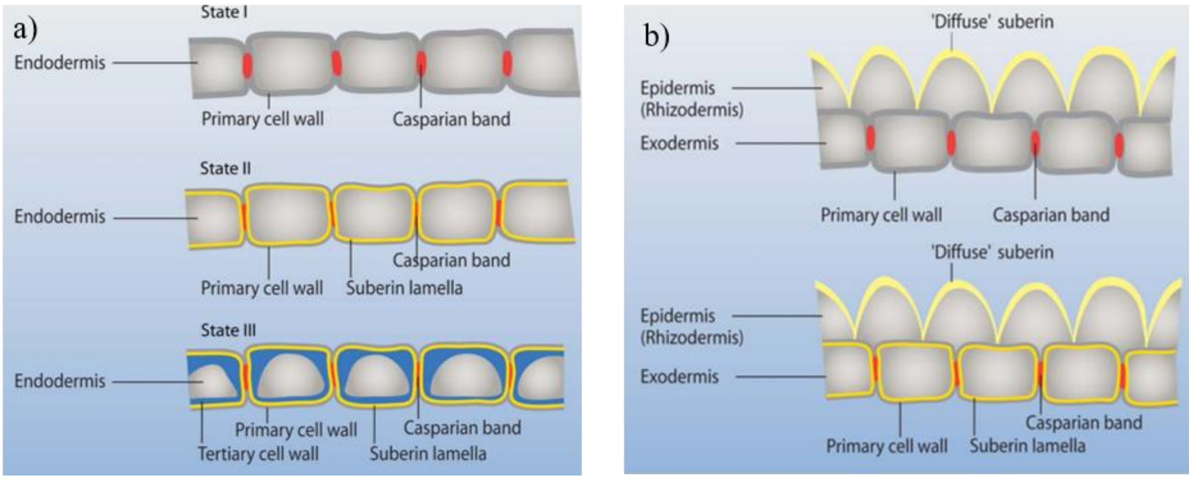


Figure 9. Development stages of endodermis (a) and exodermis (b) from (Schreiber and Franke 2011)

The stele area and xylem structures

The stele area encompasses the vascular tissues (i.e. xylem and phloem), a pericycle and in some case a pith (i.e. parenchyma cells). In particular, the xylem is a complex plant tissue specialised in transport of water and nutrients and will be extensively covered here. It comprises four cell types: fibers, parenchyma cells and two types of conducting cells (i.e. tracheids and vessel elements) (Fig. 10). Conducting cells and fibers are non-living cells that have lost their protoplast and developed thick lignified secondary walls. Xylem fibers provide support to the plant due to its lignified cell walls but do not conduct any solution. Parenchyma cells are used to store carbohydrates (e.g. starch) and to convey water and solutes into and out of the conducting cells. Due to the lack of protoplast, conducting cells form a low resistance conduit for water, nutrients and root chemical signals. Vessel elements are usually longer and wider than tracheids and perforated at both ends and sometimes along their side, whereas tracheids are perforated and tapered. However, some thin un-lignified areas called pits, remains along the vessel wall. They consist of a primary wall and a middle lamella which allow the passage of water but prevent air entry within the vessel. By aligning end to end, vessel elements form a continuous conduit commonly called a xylem vessels, whereas tracheids are not aligned and thus discontinuous. These anatomical characteristics make tracheids less efficient at conducting solution compared to vessels elements (Kirkham 2014;Nobel, 2009). Hereafter, the term xylem vessels which include both vessels elements and tracheids will be used for simplicity. There is high inter- and intra-species variability in terms of xylem vessel length, size and number (Yambao et al. 1992; Purushothaman et al. 2013; Kirkham 2014; Kumar et al. 2014). Root type and diameter are also a key determinants of xylem vessel density (Vercambre et al. 2002). Xylem tissues are usually classified into two categories depending on their level of maturation. Primary xylem vessel form from pro-cambium of apical meristem and are called protoxylem. They are very narrow vessels and are not likely to conduct any water or solutes. Later on, protoxylem vessel differentiate into wider vessels called early or late metaxylem vessels, depending on their position within the roots and stage of maturation. For plants having secondary growth (see section "Root structure evolution and maturation"), secondary xylem vessels are produced from the lateral meristem called the vascular cambium. Due to secondary growth, xylem vessel number in roots of dicotyledonous crops increases with age and were found to be more variable than in monocotyledonous crops. Xylem vessel number, maturation speed and distance from the root tip is quite variable between species (Mccully 1995) and further controlled by environmental factors (e.g. soil moisture) (Lovisolo and Schubert 1998; Henry et al. 2012; Prince et al. 2018).

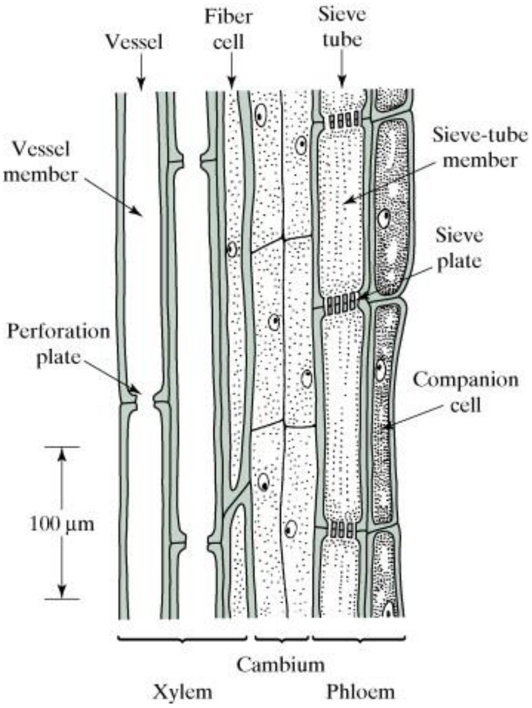


Figure 10. Schematic representation of xylem and phloem vessels in a stem from (Nobel 2009). Note. Tracheid are not represented

Linking all cell layers: Plasmodesmata

During division and differentiation in the meristematic region, plasmodesmata are formed, joining all adjacent cells across the cortex and stele. Therefore, these small channels which traverse the cell wall, interconnects cytoplasm of adjacent cells (Robards and Clarkson 1976). They form a cytoplasmic continuum commonly called the symplast, which in the root, link cells from the epidermis until the xylem parenchyma. Plasmodesmata are usually absent in cells with a secondary cell wall, although openings remains (Nobel, 2009). Juniper and Barlow, (1969), found that there are many more plasmodesmata in transverse walls than across longitudinal walls and that their density was much higher in dividing and expanding tissues. Although seen as static pores for a long time, the structure and frequency of plasmodesmata evolved over time giving plants the capacity to increase or decrease the connectivity of its tissues, see review by Roberts et al. (2003).

Root structure evolution and maturation

Root tissue maturation usually refers to the suberization of the exo- or endodermis and to the maturation of the xylem vessels that have important functional implications and have previously been covered. However, other maturation processes occur. This section focuses on particular root anatomical modifications under the form of (1) secondary root growth that occurs in most dicotyledonous species, (2) the development of root cortical aerenchyma and (3) root cortical senescence.

Secondary root growth occurs *via* radial expansion of the cambium (i.e. secondary meristems) in opposition to primary root growth which takes place at the root tip. Radial expansion starts from procambium tissues located right below the pericycle which then expand radially and destroy primary root tissues (i.e. cortex, exodermis and endodermis) (Gambetta et al. 2013). Roots that have grown radially usually have a periderm in their outer layer, secondary parenchyma cells and numerous xylem vessels (Fig. 11). This is particularly noticeable along alfalfa roots, as older roots (Fig. 11a) have a higher level of secondary growth compared to younger roots (Fig. 11b). The development of secondary metaxylem vessels from the vascular cambium considerably increases the axial transport of water at the root level (Steudle and Peterson 1998). In contrast, radial transport of water and nutrients is reduced by secondary growth due to deposition of suberin in cell walls (Gambetta et al. 2013). In addition, secondary root growth could have important implication in terms of metabolic cost, and lower levels of secondary root growth were found advantageous under phosphorus deficiency, see section "Rhizoeconomics".

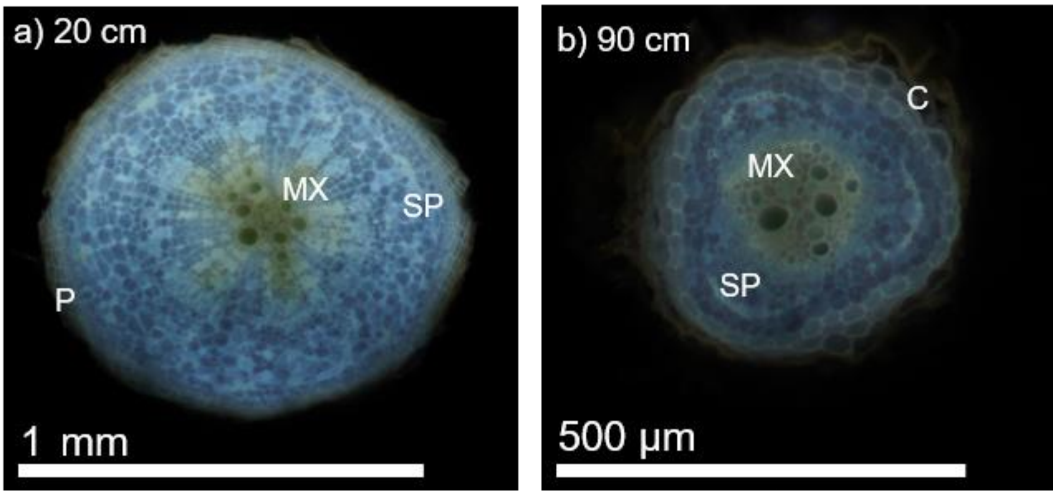


Figure 11. Cross-sections of axial roots of alfalfa presenting differences in terms of root secondary growth. Roots were sampled at 20 cm (a) and 90 cm (b) from the root-shoot junction along the same root grown in mesocosm (see paper for methodological details). Annotations refers respectively to Periderm (P), MetaXylem vessel (MX), Secondary Parenchyma (SP), and Cortex area (C)

Root cortical aerenchyma is formed through a programmed cell death that transforms living cortical cell into airspace (Fig. 12). The primary role of aerenchyma is to allow the circulation of oxygen along the roots under hypoxic conditions. Aerenchyma formation is usually associated with waterlogged conditions (Mano and Omori 2013) and is very common in a crop like rice (Colmer 2003). Nonetheless, phosphorus (Fan et al. 2007) and nitrogen deficiency (Saengwilai et al. 2014) conditions were found to increase aerenchyma formation in roots. In fact, plants with less living cortical tissue were found to have lower respirational costs and to be advantageous under low nutrient and water availability (Jaramillo et al. 2013; Galindo-Castañeda et al. 2018), see section "Rhizoeconomics". However, aerenchyma formation was found to reduce root radial hydraulic conductivity which could have important implications for the plant water status (Fan et al. 2007). High phenotypic variation in the formation of aerenchyma between cultivars offers promising opportunities for breeding of cultivars with improved drought and low nitrogen level tolerance (Mano and Omori 2013; Chimungu et al. 2015), see section "Rhizoeconomics".

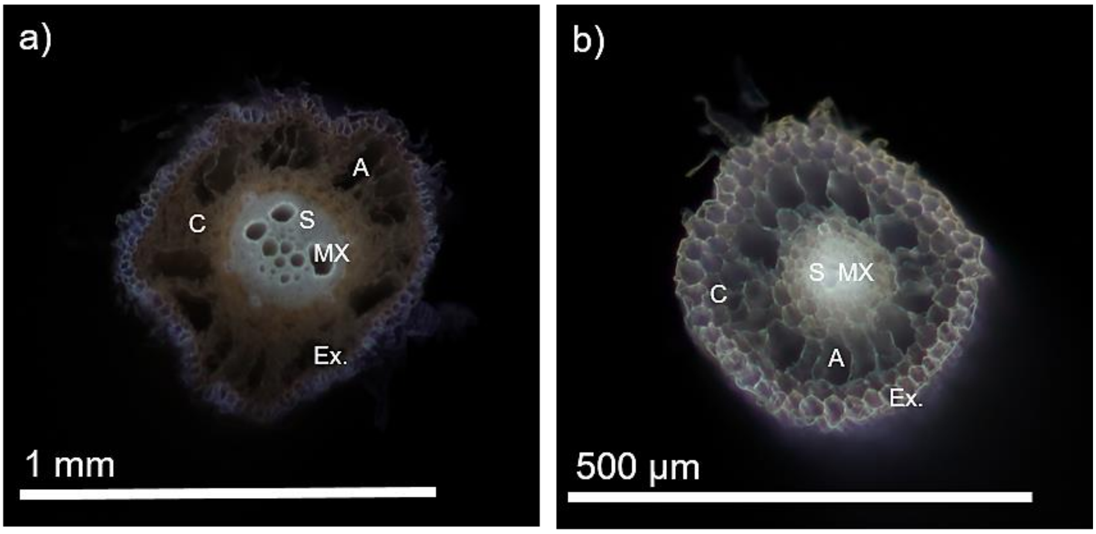


Figure 12. Cross-sections of axial (a) and respective lateral root (b) of chicory (Cichorium intybus) grown in rhizoboxes as part of this study. Cross section were obtained by laser ablation tomography. Annotations refers respectively to Exodermis (Ex.), MetaXylem vessel (MX), Cortex area (C), Stele area (S), Aerenchyma (A)

Root cortical senescence is also a programmed cell death that affect cortical cells of many plant species. Root cortical senescence is a process that is supposed to occur principally in monocotyledonous species as secondary root growth will destroy the cortex in dicotyledonous species (Fig. 13). Root cortical senescence occurs across all root types except in short lived fine lateral roots and within 2 mm from the root apex (Schneider and Lynch 2017). During this process, the cortical cells become anucleated, starting at the external root cortex and progressing inward. Root cortical senescence is a complex process that varies across plant species and growing environment but little is known empirically, see review by Schneider and Lynch (2017). Like, aerenchyma by destroying living cortical tissues, root cortical senescence reduces the metabolic cost of roots (i.e. root respiration) and drastically reduce the radial transport (Schneider et al. 2017). The fact that (1) root cortical senescence tend to be correlated to increased suberin accumulation in the endodermis and (2) root cortical senescence does not occur in lateral roots suggest that it seems to be a process that seal-off the older part of the root system. Such a process could be beneficial to avoid water and oxygen leakage, and conserve the soil water due to higher radial resistance while reducing the root metabolic cost, see section "Role of hydraulic resistance on whole plant functioning".

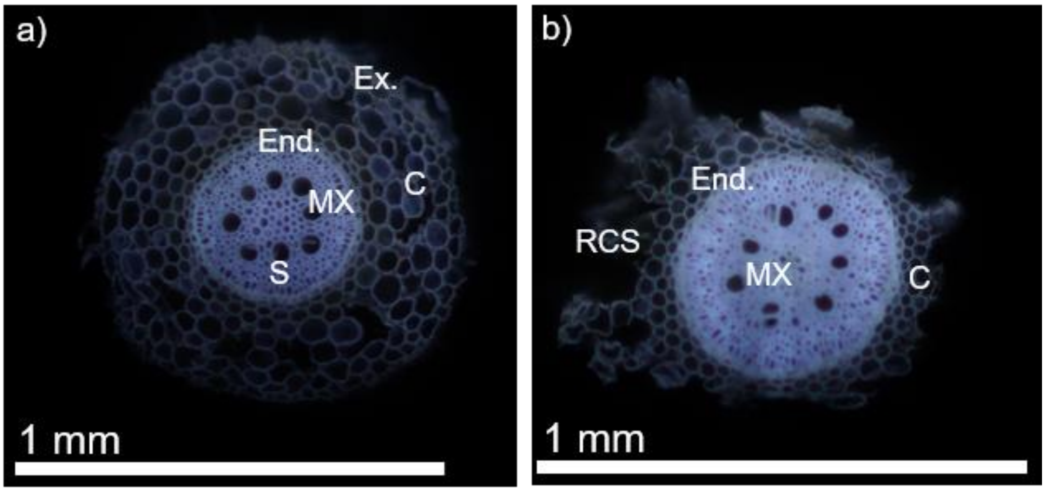


Figure 13. Cross-sections of axial roots of intermediate wheatgrass presenting differences in terms of root cortical senescence. Roots were sampled at 20 cm (a) and 10 cm (b) from the root-shoot junction on two different plants grown in mesocosm (see paper for methodological details). Annotations refers respectively to Exodermis (Ex.), Endodermis (End.) MetaXylem vessel (MX), Cortex area (C), Stele area (S) and Root Cortical Senescence (RCS)

Water flow along the soil-plant-atmosphere continuum

The transport of water in plants has long been compared to the flow of electrons in an electrical circuit where the velocity is proportional to the difference in potential and to the resistance (Van Den Honert 1948). Following this analogy, the volume of water transported across a single plant would be proportional to the difference in water potential between the soil and the leaf and to hydraulic resistances encountered in the root, stem and leaf (Landsberg and Fowkes 1978). For this purpose, plant physiologists introduced the definition of hydraulic conductivity of a plant tissue, which reflect the ease with which a fluid (i.e. sap) can move through it and is inversely proportional to the resistances encountered along the pathway. Before reaching the atmosphere, water has to flow within the soil to the vicinity of the root, then through the root to the shoots and then evaporate into the atmosphere. Therefore a lot of hydraulic resistances occur. This section covers the major determinants behind water movement along the soil-plant-atmosphere continuum with major emphasis on the root system functioning.

Water movement in the soil

Soils vary greatly in terms of particle size distribution. Following the USDA classification, soil particles are classified according to their size as follow: Gravel (> 2 mm) Sand (from 2 mm to 50 µm), Silt (from 50 to 2 µm) and Clay (< 2 µm). Between particles, pores of different size and shape may contain air, water or both and soil porosity usually represent between 40 to 60% of the total soil volume. For simplicity only the effect of particle size distribution on soil porosity is presented here but multiple factors influences soil pores and structures such as the crop type, soil management or the activity of earthworms, see review by Kautz (2014). When a soil is saturated with water all the pores are filled with water and the volume of water stored is equal to the volume of the total porosity. However, when the soil dries, soil pores provide many air-liquid interfaces, which due to surface tension lead to tension in the water or a negative hydrostatic pressure, also called the soil pressure potential. The smaller the pore size the higher the pressure potential will be. Water in the soil also have an osmotic potential depending on the amount of solutes dissolved in the water and a gravitational potential representing the gravity at a given height relative to a reference point (i.e. the reference point commonly set as the ground surface or the groundwater level). The soil water potential is used to represent the force at which water is held in the soil and corresponds to the sum of the soil pressure, osmotic and gravitational potentials. Hence, soil water content and soil water potential are correlated and the nature of their relationship is usually described by the soil water retention curve (Fig. 14). Plant physiologists have set two main soil water potential levels to consider, the field capacity (i.e. pF=1.8 or -0.03 MPa) which represents the amount of water in a soil after the surface water has drained and the permanent wilting point (i.e. pF 4.2 or -1.5 MPa) which represents the amount of water in a soil when the plant cannot physiologically extract water anymore. Along with a decrease in soil water content the soil water potential will decrease and plants must increase the force they apply to extract the remaining water. Water movement through soil is driven by a gradient of hydrostatic pressure and is commonly describe by the Darcy's law (Eq. 1):

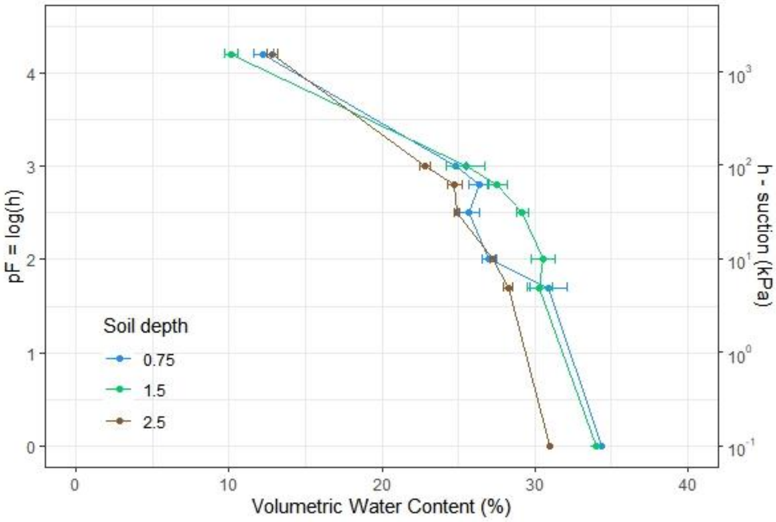


Figure 14. Soil water retention curve measured at 0.75, 1.5 and 2.5 m soil depth (points - average value ± standard error. Measured using a pressure plate apparatus and sand bed. From (Clément et al. In preparation)

Equation 1. $$Q = K * A * \Delta H$$

Where Q refers to the water flow (m^3/s), K the hydraulic conductivity of the soil (m/sec), A the cross-sectional area through which water moves and ΔH the hydraulic pressure gradient. Hence, when the soil is drying the soil pressure potential decreases and the large pores will become air-filled, reducing the soil hydraulic conductivity. As long as the soil hydraulic conductivity towards roots remains higher than the plant hydraulic conductivity, water absorbed at the root vicinity will be replenished and will compensate the plant extraction. If not, the root water uptake will be limited by the soil hydraulic conductance, as discussed by Lobet et al. (2014).

Maintaining hydraulic continuity at the root-soil interface

Hydraulic continuity from the soil to the roots must persist in order to support plant transpiration. Apart from hydraulic resistances intrinsic to the soil or the plant, a critical zone where high resistances to water flow could occur is at the root-soil interface. The rhizosphere, the volume of soil influenced by roots, is complex due to interactions between soil, root, water and microorganisms which give it specific physicochemical and structural properties (Hinsinger et al. 2009). The two main phenomena that could decrease the hydraulic conductivity within the rhizosphere are (1) poor root-soil contact and (2) a drop of water potential due to drying. Other processes can occur such as salt accumulation at the root surface creating osmotic stress but will be kept out of this review as they are considered to be specific cases. At the root-soil interface, main processes that affect the rhizosphere hydraulic properties are (1) the secretion of mucilage from the root, (2) the accumulation of fungal hyphae and bacteria, (3) the presence of root hairs and (4) the creation of air gaps due to shrinking/swelling of the soil or root. Mucilage excretion occurs at the root tip and increases soil aggregation around roots (also called rhizosheats, (Fig. 15a)), especially under dry conditions (Watt et al. 1994). Improved soil aggregation surrounding roots was also found around roots inoculated with the bacteria *Pantoea aglomerans* (Amellal et al. 1998). However, the effect on the rhizosphere water retention properties were not tested. Inoculation with fungi also increases micro-aggregate stability at the root-soil interface (Augé 2001). Therefore, fungi and bacteria might improve the wettability of the rhizosphere soil as soil with greater aggregate stability and improved porosity usually present a higher water holding capacity. However, little

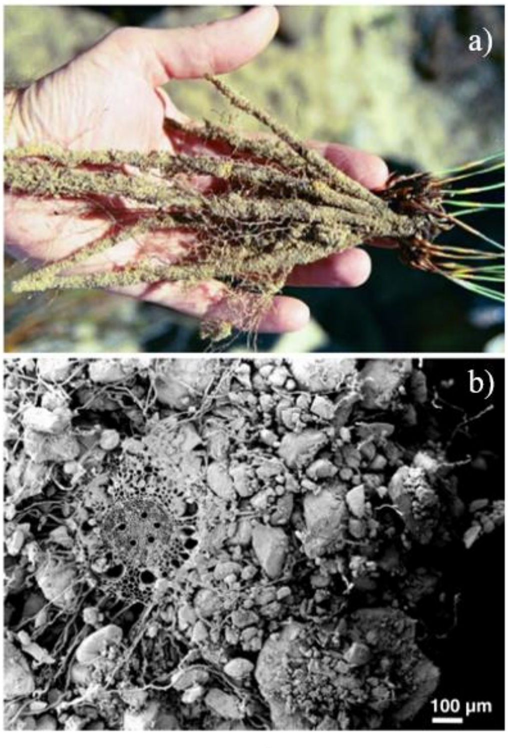


Figure 15. (a) Soil aggregation (i.e. rhizosheats) around roots of Lyginia barbata (b) and root of barley (Hordeum vulgare L.) growing in the field with root hairs crossing macropores from (Hinsinger et al. 2009)

is known on the direct influence of fungi on root water uptake, especially because of the difficulty of separating direct and indirect effects associated with mycorrhizal fungi (e.g. enhance nutrient supply). In addition, Mccully and Boyer (1997) showed that mucilage of maize root could absorb significant amount of water. This could explain why Carminati et al. (2010) found that the rhizosphere around roots of lupine had higher water holding capacity and remained wetter than the bulk soil under drying. The higher moisture content in the rhizosphere improves the flow of water from dry soil to the roots (Ahmed et al. 2014). A recent review concluded that mucilage by absorbing water, decreasing the surface tension and increasing the viscosity of the soil solution, allow the creation of "liquid bridges" throughout the rhizosphere at low water potential and thus facilitates water uptake under drying (Carminati et al. 2017a). However, a study by Carminati (2013) showed that mucilage could turn hydrophobic upon drying and that rewetting of the rhizosphere in young root segments was faster compared to old root segments. In fact, the level of hydrophobicity of the rhizosphere depends on the mucilage concentration and the soil type (Ahmed et al. 2016a). Such a phenomenon will reduce root water uptake but could also isolate roots from their environment and prevent water leakage from the root to the soil as discussed by Zarebanadkouki et al. (2018). Poor root-soil contact can also emerge under drying as soil and roots can shrink (Carminati et al. 2013) or due to roots growing in macropores (White and Kirkegaard 2010). Root hairs have long been recognized to increase the volume of soil being explored and they could contribute up to 70 and 90% of the total root surface area. They are particularly noticeable, and beneficial, when a gap exist at the root-soil interface (Fig. 15b). Apart from their role in nutrient uptake (Bates and Lynch 2001), root hairs significantly facilitate the uptake of water by increasing the volume of soil being explored (Segal et al. 2008) and by reducing the drop of soil water potential at the root surface under dry conditions (Carminati et al. 2017b). However, root hair formation differ between plant species, root type and environmental conditions (Nestler et al. 2016) and study on root hairs under field conditions remains particularly challenging. In the end, plants poses a range of processes helping them maintain hydraulic continuity from the soil to the roots. While some processes increase the hydraulic resistance (e.g. hydrophobicity, air gaps), they still might not necessarily be disadvantageous. In fact, plasticity of the rhizosphere hydraulic properties seems a key function for efficient water use. Indeed, by having portions of the root system well connected to moist soil while other parts remain isolated from the soil due to rhizosphere hydrophobicity or air gaps, the rhizosphere contributes to the partitioning of the plant water uptake (Carminati and Vetterlein 2013). Differences in hydraulic resistances along the root system play a key role in regulating the distribution of plant water uptake, especially from deep soil layers, see section "Role of the hydraulic resistance on whole plant functioning".

The driving force and leaf water flow

Water moves passively through roots and plants, from the soil to the atmosphere, along a pressure gradient set up by transpiration and osmotic forces (Steudle and Peterson 1998). Transpiration refers to the process of evaporation of water molecules from the leaf mesophyll cell into the intercellular space. Once there, water will reach the atmosphere *via* the stomata pore. Each water molecule that evaporates from the cell wall is directly replaced within the wall due to cohesive forces, generated by hydrogen bonding, creating a suction force that is transmitted along the xylem and then to the roots. This process creates a hydraulic gradient allowing water to move along the so called Soil-Plant-Atmosphere Continuum (Fig. 16), see review of McElrone et al. (2013).

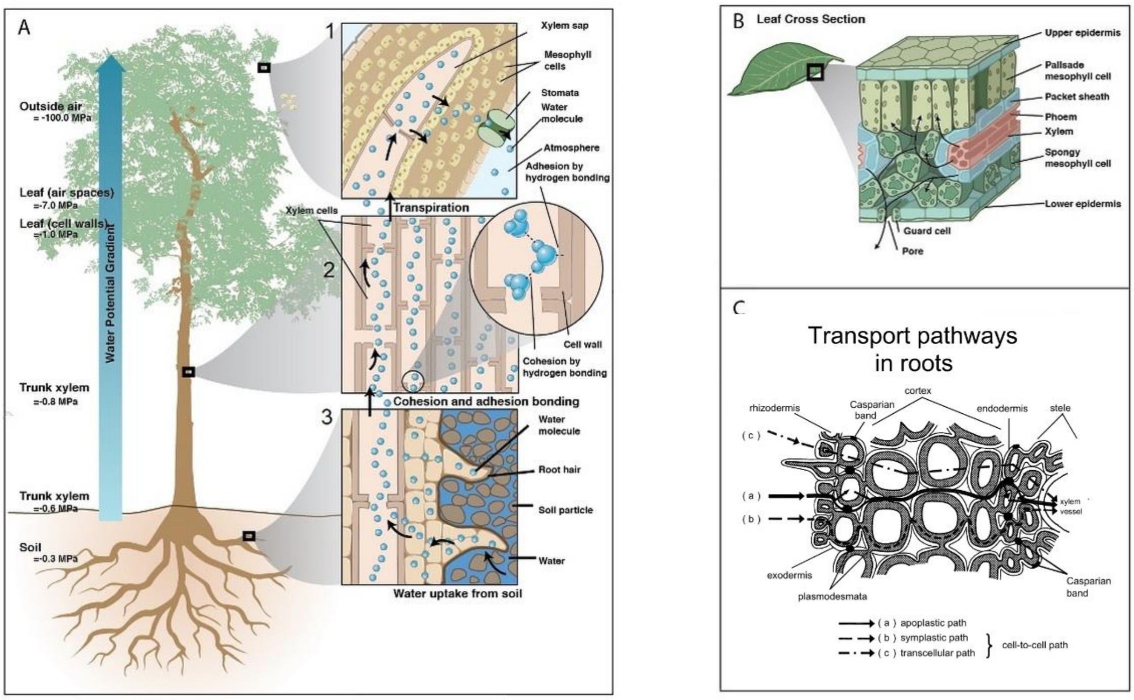


Figure 16. Schematic drawing of the water transport along the soil-plant-atmosphere continuum from (McElrone et al. 2013) (A) Water movement from the soil, through the plant and to the air. (1) Transpiration: evaporation of water from the leaf creating tension and movement of water (2) through the xylem and (3) radially through the roots and then to the soil. (B) Radial water pathway at the leaf level. (C) Radial water pathways across a root segment. Water can move radially via (a) the apoplastic path (i.e. through the apoplast) (b) the symplastic path (i.e. through the cytoplasmic continuum) or (c) the transcellular path (i.e. through the cytoplasm and vacuole) from (Steudle 2000a)

The evaporation rate within the intercellular space is controlled by the openings and closing of stomata, further detailed in section "Leaf hydraulic resistance". In addition, the active uptake of nutrients in the root creates an osmotic pressure across cell membrane that also drives water across plant cells. Hence, water and nutrient transport are, to some extent, coupled and stomatal opening and active uptake of nutrients constitute the only two active mechanisms involved in the creation of a water potential gradient, necessary to plant water acquisition. However, during periods with transpiration the hydraulic gradient created is much higher than the osmotic gradient, this latter playing a role mostly under low or without transpiration.

The root water pathway

In the root, water flow radially across the cortex and axially within the stele. Hence, two types of flow apply, a radial and an axial flow with respective conductivities and resistances. Each specific tissue and cells encountered along the pathway have individual hydraulic properties influencing the root water conductance, which are described below.

Axial conductivity and resistances

Following the pressure gradient, setup by transpiration, tension will rise into the xylem vessels engendering water and nutrients to flow axially along the root. The axial conductivity within a xylem vessels is theoretically estimated using the Poiseuille law (Nobel, 2009 p.448). This law states that the volume of fluid moving per time across a cylinder is proportional to the fourth power of its radius and the hydraulic pressure gradient that exists along the section, see equation 2:

$$Equation\ 2: \qquad v = -\left(\frac{\pi r^4}{8n}\right)\left(\frac{\delta P}{\delta X}\right)$$

where v is the rate of volume movement (m^3/s), n the solution viscosity (kg/m/s), r the radius of the cylinder (m) and (δP/δX) the negative gradient of the hydrostatic pressure. Note that the Poiseuille law does not take in consideration the friction resistances that occur along the cylinder. At the root level, the axial conductivity depends on the development and maturation of xylem vessels (Lovisolo and Schubert 1998; Vercambre et al. 2002). Along the axial pathway, hydraulic resistances depend on the length, size, structure and degree of interconnection of the xylem vessels (Sellin 1993; Lobet et al. 2014). Thus, axial conductivity decreases toward the root tip due to high axial resistances caused by immature xylem vessels (Frensch and Steudle 1989; Steudle and Peterson 1998). In dicotyledonous species, development of secondary xylem vessels could increase hydraulic conductivity by up to a factor 1000 (Vercambre et al. 2002) while in monocotyledonous species, xylem vessel number and size are determined at a much earlier stage and remain constant throughout the plant lifetime (Watt et al. 2008). Within xylem vessels, water under tension fall below its vapor pressure and continuity along the water column is determinant to maintain hydraulic conductivity and water flow. However, under very high tension (e.g. water stress, freezing), air dissolved in water will expand and fill the xylem vessels in a process called cavitation. Embolized (i.e. cavitated) xylem vessels are not conducting in this state as the water column is disrupted and the overall plant axial conductivity is decreased. However, recovery from embolized vessels exist (e.g. root pressure for monocotyledonous and secondary xylem vessels formation for dicotyledonous, see review by Brodersen and McElrone (2013)). Therefore, xylem vessels exist in a state of equilibrium between cavitation and refilling with oscillation from one to another being more frequent than expected (Holbrook and Zwieniecki 2005). Such, physiological threat to plant water supply play a key role in plant evolution and water related strategies (Tyree and Sperry 1989). Along the axial pathway, water move radially or longitudinally from one xylem cell to another through pits, which constitute the main resistance along the axial pathway (McElrone et al. 2013). Adhesion, i.e. the attraction force between a liquid and a solid phase, occurs at the interface between water and xylem cell walls. Due to surface tension, water inside a xylem vessel will rise along its inner wall, the smaller the vessel, the higher the water column will rise, and this phenomenon is called capillarity. Gravity will also apply a downward oriented force on the water column so the extent of rise (h) created by these two forces can be calculated by solving the equation from (Nobel, 2009):

$$Equation\ 3: \qquad \pi r^2 h\rho g = 2\pi r\sigma \cos\alpha \quad thus\ h = \frac{2\sigma\cos\alpha}{r\rho g}$$

where G is the gravitational force, r the radius of the cylinder (cm), h the height of the water column (cm), ρ the density of the fluid, σ the surface tension of the fluid and α the contact angle at the water-air interface. Gravitational forces, by creating resistance towards the upward movement of water increase the risk of cavitation. In consequence, deeper roots have to cope with higher gravitational resistance.

Radial conductivity and resistances

To understand root radial flow, it is necessary to understand how water moves at the cell level and across cell layers. Plant cells are commonly known to be constituted of, from outside to inside, a cell wall < plasma membrane < cytoplasm < vacuole (Fig. 16c). As previously mentioned, cytoplasm of nearby cells are interconnected by microscopic channels called plasmodesmata. Overall, they form a "cytoplasmic continuum" called the symplast whereas the cell area outside the plasma membrane is called the apoplast. Considering that water can easily cross membranes *via* water channel proteins called aquaporins, water can follow three pathways at the cell level: (1) the "Apoplastic pathway" within the apoplast i.e. around the cell protoplasts, (2) the "Symplastic pathway" within the symplast and through plasmodesmata and (3) the "Transcellular pathway" or "vacuolar pathway" by crossing cells membranes (i.e. plasmalemma & tonoplast) (Fig. 16c) (Steudle and Peterson 1998; Steudle 2000a). The symplastic and the transcellular pathway are regrouped under the term "cell-to-cell pathway" as they cannot be separated experimentally. At the tissue level, these pathways are not mutually exclusive and radial movement of water should be seen as a combination of these different paths and is usually described as the composite transport model of water (See section "Contribution of respective pathways").

- *Radial conductivity*

Along the radial pathway, the composite structure of root is usually simplified as a series of concentric cell layers containing apoplastic and cell-to-cell hydraulic resistances working in parallel. Main resistances along the cell-to-cell pathway lie at the cell membrane (Kirkham 2014). In particular, the plasma membrane has a lower permeability than the tonoplast and thus constitute the main limiting barrier to water flow along this pathway, as discussed by Javot and Maurel (2002). This confirm why aquaporin activity could account for 21% to 90% of root hydraulic conductance depending on the species (Javot and Maurel 2002; Tyerman et al. 2002). Although the main radial resistances to water flow come from living tissue (i.e. non-vascular tissue), the xylem vessel walls also create resistance for about 10-30% of the total radial hydraulic resistances. (Peterson and Steudle 1993). As a simplification, it has been proposed that the radial diffusion time across a root is proportional to the second power of the root radius (Ahmed et al. 2016b). In fact, radial conductance is inversely proportional to the length of the flow path (Bramley et al. 2009), aquaporin activity (Steudle and Frensch 1996) and the degree of maturation of the endodermis and exodermis. This was confirmed by Zarebanadkouki et al. (2016) who found that radial conductivity increases toward the root tip where maturity of casparian bands and suberin lamellae decreases. Particular cases occur in roots having aerenchyma or root cortical senescence as radial hydraulic conductivity decreases due to the reduction in living cortical cells (Hu et al. 2014).

- *Hydraulic resistances along the apoplastic pathway (physical barrier)*

Along the apoplastic pathway, water, solutes and dissolved gases can move freely from the plant to the soil as flow along cell walls does not have selective properties. In response, plants developed means to regulate their water and nutrient transport along this pathway. Regulation is achieved by modifying the cell wall of exodermal and endodermal cells, forming hydrophobic barriers called casparian band or suberin lamellae, see section "The endodermis and exodermis: Two specialized cortical cell layers". If sufficiently hydrophobic, casparian bands could effectively block the apoplastic pathway while suberin lamellae could block access to the plasma membrane by forming a secondary cell wall. Such anatomical modifications are irreversible and decreases radial flow along the apoplastic pathway (Sanderson 1983; Zimmermann et al. 2000; Baxter et al. 2009). The level of resistance to solute and water flow depends on the degree of maturation of these two cell layers and the density of passage cells. Development and maturation of the endodermis and exodermis depends on the plant species (Baxter et al. 2009; Watanabe et al. 2013), type and diameter of roots (Tylová et al. 2017), growth conditions and nature of abiotic stress (Enstone et al. 2003; Vandeleur et al. 2009). Indeed, suberization of endodermis and exodermis usually increase with age and under drought, salt and water logging stress (Steudle 2000b; Enstone et al. 2003; Vandeleur et al. 2009; Schreiber and Franke 2011). Suberization of the endodermis was also found to increase with increased root cortical senescence (Schneider et al. 2017). Furthermore, genetic material, root order, soil nutrient status or soil mechanical impedance are others factors that affect the development of endodermis and exodermis. In fact, much remains to be learned concerning the accumulation and maturation of suberin in plant tissues under heterogeneous field conditions (Watanabe et al. 2013).

- *Hydraulic resistances along the cell-to-cell pathway (aquaporin regulations)*

Along the symplastic pathway, the flow of water occurs by diffusion through plasmodesmata (Roberts et al. 2003). The distribution, density and structure of plasmodesmata within cell walls regulate the water conductivity along this pathway (Juniper and Barlow 1969; Couvreur et al. 2018). Along the transcellular pathway, a more flexible control is likely to be done *via* aquaporins located in the plasma membrane and tonoplast of most plant tissues (Maurel 1997; Maurel et al. 2010). Aquaporins are formed of six transmembrane α-helices, which are disposed in a way to create a pore allowing water, dissolved gasses and small uncharged solutes to move across the cell membrane, which by nature is an impermeable barrier to these molecules (Maurel et al. 2008; Chaumont and Tyerman 2014). Regulation of membrane permeability can be done by changing aquaporin density or activity, which could be achieved *via* adjustments of gene expression, post-translational mechanisms, aquaporin relocation or closing of the pore. Indeed, many post-translational mechanisms (e.g. phosphorylation, methylation) have been illustrated to affect the channel activity and density in the cell membrane allowing rapid regulation of cell and tissue water flow properties (Chaumont and Tyerman 2014; Vandeleur et al. 2014). Displacement of aquaporin from one membrane to another can also occur, inducing a rapid change in protein abundance (Luu and Maurel 2013; Chaumont and Tyerman 2014). Finally, closing of aquaporins is regulated by hormonal signalling (e.g. ABA, salicylic acid, ethylene) (Parent et al. 2009), the osmotic gradient (Carvajal et al. 1996; Tyerman et al. 2002), pressure signals i.e. change in transpiration rate or leaf excision/wound or change in cytosolic pH (Tournaire-Roux et al. 2003). Higher aquaporin densities were observed in cells where water flow is concentrated (i.e. endodermis, exodermis, stele, around xylem vessels) (Maurel et al. 2008; Chaumont and Tyerman 2014). Their expression commonly follows a diurnal cycle, with higher expression during the day when evaporative demand is the highest (McElrone et al. 2007; Sakurai-Ishikawa et al. 2011; Takase et al. 2011; Hachez et al. 2012). By consequence, they play a key role in maintaining cell turgor in expanding tissues (Maurel et al. 2008). Aquaporin expression is usually downregulated by cold temperature and hypoxic conditions (Maurel et al. 2008) but some cold- or anoxic tolerant plants show higher expression of certain aquaporin isoforms under anoxic conditions (Choi and Roberts 2007) and cold temperature (Aroca et al. 2005). Overall, they constitute the major regulator and resistance to water flow along the cell-to-cell pathway and play a key role in whole plant water fluxes, especially under drought (Grondin et al. 2016).

- *Contribution of respective pathways*

The electrical analogy of water flow through plants and roots remains a simplified representation. It does not hold as the different pressures (i.e. hydrostatic and osmotic) and potentials driving the flux of water does not affect the different water pathways in a similar way. Indeed, as a cell wall is not a selective structure, water along the apoplastic pathway is driven by hydraulic forces (Jones et al. 1988) while water along the cell-to-cell pathway is driven by both osmotic and hydraulic forces due to the selectivity of plasma membrane. Along this pathway water has to cross many membranes resulting in much higher hydraulic resistances than under the apoplastic pathway. Furthermore, the concentration of solute fluctuates across the apoplast and symplast resulting in a redistribution of water along the pathways. It is also worth mentioning, that hydraulic resistances decrease as velocity increases (Steudle 2000a). Overall, when there is a hydrostatic pressure gradient (i.e. transpiration), water is transported mostly via the apoplastic pathway due to lower hydraulic resistances (i.e. fewer membranes to cross) in roots (Steudle 2000a). Nonetheless, osmotic forces are not negligible. Contribution of hydraulic and osmotic forces to water flow is known to be quite variable across plant species and root type (Knipfer and Fricke 2011). For example, in maize (*Zea mays*) and common beans (*Phaseolus vulgaris*) water predominantly moves due to hydraulic forces, while in barley (*Hordeum distichon*) and lima beans (*Phaseolus coccineus*) hydraulic and osmotic forces contribute nearly equally to water flow (Steudle 2000a). The variability is predicted to be due to changes in membrane permeability or to the tightness of the casparian bands. In the absence or at low transpiration rate, water moves preferentially through the cell-to-cell pathway following the osmotic gradient created by the active uptake of nutrients across membranes.

Leaf hydraulic resistance

The leaf hydraulic architecture is usually separated into a low-resistance pathway (i.e. along the xylem vessels) and a high-resistance pathway across the bundle sheath cells, leaf mesophyll cells and out to the air (Fig. 16b). Along the latter pathway, movement of gaseous water through stomata constitutes the highest resistance to water flow due to the low diffusion of water in the air and limited surface area of stomata openings. Stomata are pores located at the epidermis of leaves and are bordered by guard cells, which under the pressure and tensions from adjacent epidermal cells, regulate the size of the stomatal opening. Hence, stomatal regulation plays a critical role on shoot and root hydraulics and on the plant water status. At a similar stomata opening, lower air humidity (i.e. high vapor pressure deficit (VPD)) will result in a high transpiration rate and thus higher hydraulic forces applying along the plant xylem. It is now well known that stomatal aperture is sensitive to VPD and recent findings showed high genotypic variations in the response of stomata to change in VPD among crop species and cultivars (Vadez et al. 2014). Stomatal aperture is controlled by multiple and complex chemical and hydraulic signals (Tardieu and Davies 1983). Among others, abscicic acid (ABA), CO_2 concentration, temperature, light intensity and plant hydraulic conductance constitutes some of the signals involved that occurs in synergy (Comstock 2002; Christmann et al. 2007). Although physiologically important, these complex regulatory mechanisms have been excluded from this review as understanding of these synergies is still at an early stage and often applies to controlled conditions. Nonetheless, hydraulic resistances within the leaf contribute, on average, to more than 30% of the total resistances to water flow through the plant (Sack and Holbrook 2006,Park Nobel, 2005). Leaf hydraulic conductance, and by analogy resistances, are highly variable across plant species, age and growing conditions especially due to differences in venation, leaf anatomy, or stomatal response to environmental conditions (for a review see Prado and Maurel (2013).

Role of the hydraulic resistance on whole plant functioning

Leaf transpiration determines the suction force that is exerted on the plant and root system. Therefore, resistance to water diffusion outside of the stomatal chamber have considerable influence on the whole plant water uptake. The distribution of resistance along the water path from the soil to the plant determines the distribution of water uptake from the soil. In simple words, when topsoil is very dry, water uptake will occur below that soil layer as long as the plant internal resistances are lower than the transpirational force. The root system represent the second largest resistance to water flow at the plant level, the first being the diffusion of water through the stomata (Steudle and Peterson 1998). In this section, special emphasis is placed on transpiration and root system hydraulic resistances but internal leaf and stem resistances do occur.

- *Transpiration regulation*

Plants have been classified in two categories based on their transpirational strategy, (1) anisohydric plants which tolerate fluctuation of leaf water potential and (2) isohydric plants which maintain a constant leaf water potential. Therefore, under drought isohydric plants tend to reduce their stomatal conductance to maintain a constant leaf water potential whereas anisohydric plants keep them open inducing a decrease in leaf water potential (Sade et al. 2012). When comparing stomatal conductance measurements from this study (Fig. 17), to observations from (Tardieu and Simonneau 1998) on sunflower (anisohydric) and maize (isohydric), it seems that alfalfa tends to be anisohydric whereas intermediate wheatgrass tends to be isohydric. Indeed, alfalfa had a much higher stomatal conductance that decreases throughout the day, especially in the dry year of 2018, whereas intermediate wheatgrass tend to maintain a lower but constant stomatal conductance. While low or reduced transpiration under high vapor pressure deficit is seen as a key genotypic trait to improve transpiration efficiency (Vadez et al. 2014), reducing transpiration will reduce the hydraulic forces applied along the root xylem and thereby potentially limit deep water uptake.

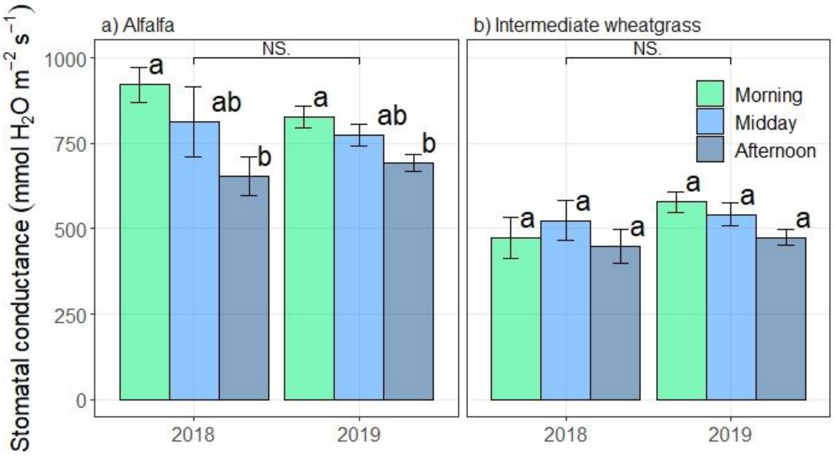


Figure 17. Daily stomatal conductance of alfalfa (a) and intermediate wheatgrass (b) during two seasons of cultivation. Data collection occurred approximately every two weeks at 8h-10h ("Morning"), 11h-13h ("Midday"), and 15h-17h ("Afternoon"). Average value ± standard errors (n_{2018}=6; n_{2019}=8). Comparisons were made between collection time and years, letters that differ indicate significant differences at ($p<0.05$). Annual differences are reported in brackets. (From, Clément et al., in preparation)

Hence, for crops with reliable deep-water access, reducing transpiration rate under high vapor pressure deficit might not be a key advantage to maximize soil water use and production. Indeed, more research is needed to understand plant transpiration and stomatal aperture response to multiple environmental factors, especially topsoil drying and increasing vapor pressure deficit (Vadez et al. 2014). In conclusion, when breeding and selecting crops toward deep-water use, focus should be done on unrestricted transpiration under both high vapor pressure deficit and dried topsoil conditions that would enable deep-water uptake when resources are available at depth. When the water resource is scarce, also from deep soil layers, there is a threshold below which water consumption becomes risky and cavitation damages induced by increased xylem tension become irreversible. Thus being anisohydric is not advantageous anymore. Understanding of the Genotype x Environment x crop Management interactions that could lead to such situations is important, see section "G x E x M".

- *Root hydraulic resistances and root water uptake distribution*

As in the leaf, the root hydraulic architecture could be separated into a low and a high resistance pathway. Indeed, radial hydraulic resistances are bigger than axial hydraulic resistances, except at the root tip (<2 mm) which is hydraulically isolated (Steudle 2000a; Zwieniecki et al. 2003; Vadez 2014). At the plant level, the distribution of root water uptake depends on the distribution of radial and axial resistances. The composite transport model of water through root allow plants to regulate their water uptake according to shoot demand and environmental factors (Steudle 2000a, b; Hose et al. 2001; Couvreur et al. 2018). This is achieved *via* the regulation of hydraulic resistances along the different pathways making roots acting as hydraulic rheostats, as proposed by Maurel et al. (2010). Such regulation could also occur within the rhizosphere soil as mentioned before. Roots, by analogy, could be seen as a "porous pipe" where the distribution of water uptake along the pipe is proportional to the ratio of axial to radial hydraulic resistances (Landsberg and Fowkes 1978; Zwieniecki et al. 2003; Zarebanadkouki et al. 2016). Therefore, hydraulic resistances regulate the flow of water within the root but also the distribution of water uptake within the root system. As shown in figure 18, for a constant axial resistance an increase in radial resistance would induce a more homogeneous root length utilisation but a reduction of actual water uptake as total resistance would increase in the root system. Inversely, at constant axial resistance but lower radial resistance a smaller part of the root length would have active uptake but total water uptake would increase. Therefore, the axial to radial resistance ratio illustrates how conductive and isolated a root segment is. The lower the ratio, the lower the conductance and the more isolated the root segment is (Zwieniecki et al. 2003).

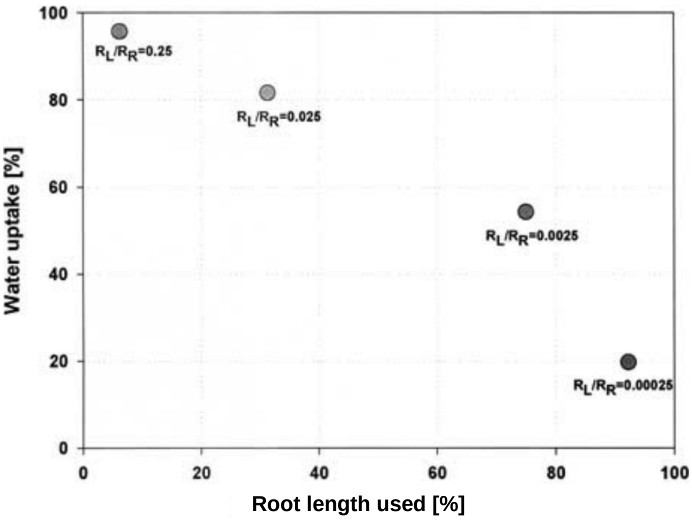


Figure 18. Relation between percentage of root length used and the percentage of water uptake for different level of radial resistances (R_R) but constant axial resistance (R_L). Water uptake refers to the percentage of water being taken up in comparison to the maximum water uptake (100%). Adapted from (Zwieniecki et al. 2003)

In consequence, without control of root radial permeability, plants would not be able to absorb water from the most distal part of their root system (i.e. the farthest and deepest) as most water would be taken, or leak out, from the most proximal part (Zwieniecki et al. 2003). For monocotyledonous species, senescence of the root cortex and suberin deposition in the exo- or endodermis are the major processes that prevent roots from drying under dry soil conditions. These processes isolate roots from dry soil environment and maintain the hydraulic connection between shoot and deeper portion of the root system. For dicotyledonous species, such functions will be ensured by highly suberized roots that have followed secondary root growth. Nonetheless, when water is available at depth, the axial resistances might still be limiting root water uptake from deep soil layers (Garrigues et al. 2006; Pierret et al. 2006).

- Root hydraulic resistances and water flow regulation

Due to the composite transport model of radial movement of water in roots, the flow of water can be finely regulated by different mechanisms. In the apoplast, without the presence of endodermal/exodermal apoplastic barriers, water would flow preferentially along the apoplastic pathway but water would also flow back toward the soil when soil water potential is lower than the xylem potential (i.e. under drought or salt stress) (Schreiber and Franke 2011). In fact, flow of water from regions of high water potential to regions of low water potential within the root system have been identified at night (i.e. in the absence of transpiration). This mechanism is called hydraulic lift (Caldwell and Richards 1998) but might be limited to the region of the root system with immature endodermis, exodermis or that have not engaged in secondary root growth (i.e.

close to root tips or fine short-lived lateral roots). When suberization of the root endodermis occurs, the apoplastic pathway is interrupted hence forcing water to flow through the cell-to-cell pathway. This latter is obviously usually rate limiting to the flow of water through roots. Aquaporins, by regulating plant water flow across membranes can rapidly change the hydraulic conductivity along the cell-to-cell pathway and play a central role when the apoplastic pathway is hindered (Maurel et al. 2010). It is not surprising that their activity has been found to favor root water uptake under drought and salt stress (Steudle 2000a; Martre et al. 2001; Lian et al. 2004; Maurel et al. 2008) and recovery after water deficit (Martre et al. 2002). Aquaporin expression has also been correlated to tissue, cell type and to the presence of apoplastic barriers (Knipfer and Fricke 2011; Hachez et al. 2012; Chaumont and Tyerman 2014). However, overexpression of aquaporins has also been reported to induce a decrease in drought resistance (Aroca and Ruiz-lozano 2012) and not all aquaporin isoforms are upregulated under abiotic stress (Tyerman et al. 2002). Altogether, the transport of water through root is tightly controlled by the extent of radial hydraulic resistances (Maurel et al. 2008, 2010; Ehlert et al. 2009; Chaumont and Tyerman 2014). Development of apoplastic barriers and the expression and activity of aquaporins are commonly described respectively as "coarse" and "fine" regulation means of plant water uptake (Steudle 2000a; Hose et al. 2001).

- *Water use strategies*

All in all, increasing root axial conductance has been proposed as a key trait for deep water uptake (Yambao et al. 1992; Wasson et al. 2012; Lynch et al. 2014). Under dry soil conditions, decrease in radial resistances *via* upregulation of deep root aquaporin activity and expression was found correlated to upregulation of hydraulic conductivity of deep roots of trees (Mclean et al. 2011; Johnson et al. 2014). Similarly, compensating aquaporin activity were shown by Ehlert et al. (2009) when inhibiting aquaporin activity in parts of a maize root system. Higher aquaporin expression under drought led to more drought tolerant cultivars of grapevine and tomato, respectively, highlighting the role that aquaporin plays in determining the plant isohydric/anisohydric threshold (Sade et al. 2009; Vandeleur et al. 2009). Hence, the extent of hydraulic resistances along the water pathway partly determines the plant water strategy i.e. water saving vs. water consuming and their isohydric threshold. Overall, greater axial and radial hydraulic conductivity is usually associated to improve drought tolerance (Wasson et al. 2012; Lobet et al. 2014). Maintaining a sealed top root system and a porous deep root system with low hydraulic resistances seems to be an interesting trait that would enable a plant to maintain deep water uptake (Zwieniecki et al. 2003). Reducing the plant isohydric threshold and understanding deep root aquaporin regulation are also important physiological traits that would favour deep-water uptake especially under heterogeneous water availability (i.e. dry topsoil conditions).

Deep roots, perennial crops and agricultural perspectives

Forward

Perennial vs annual crops, water use efficiency vs effective use of water, deep vs shallow rooted crops, it seems that the agricultural and research world is full of endless debates. The nature of these debates often originates in the lack of contextualisation in terms of environmental conditions, farming context and goal. While deep water use and perennial crops are certainly advantageous in some context, understanding the fitting landscape of such species and plant traits is not an easy task. In the first chapter the main anatomical and physiological processes involved in root growth and crop water uptake were covered. While this knowledge may be sufficient to understand water movement and uptake at the plant level, making use of this knowledge in an agricultural context requires much broader considerations. Indeed, in the field, crops face a wide range of environmental conditions that vary with time, space and crop management. Therefore, improvement of agricultural systems will come from sounds understanding of the interactions between the crop, the environment and the crop management that could lead to better performances. This second chapter aims at broadening and replacing crop growth and water uptake into agricultural context with a focus on the role and influence of the root system. Although some parts may seem far removed from the doctoral work itself, the authors believe that they remain essential to fully understand the agricultural reasoning behind this study. This chapter is not as detailed as previous chapter due to the vast range of scientific fields that it touch upon but should be seen as a call for innovative translational approaches in agricultural research that are highly needed (Passioura 2020).

Timing, efficiency and partitioning of soil resource use

Crop growth is dependent upon its phenology, nutritional supply and the environmental conditions they grow in. At the same time, organ size and distribution determine the spatial zone of resource uptake. For example, the leaf surface area determines the light interception capacity of the plant, which overall influence the crop growth and productivity (Koester et al. 2014). However, the relationship between soil resource capture and crop growth, is not linear mostly due to (1) uneven distribution and availability of soil resources (Hodge 2004), (2) carbohydrates partitioning to different organs (Weraduwage et al. 2015) or differences in resource use efficiency in time and among species, for a review see Zhu et al. (2010). For example, the efficiency of the root system to capture soil resources is coupled to root lifespan as root uptake rates decrease with age. Overall, this reminds us of the metabolic costs associated to resource uptake, crop growth and biomass production and of its feedback effect on the overall crop functioning. Researchers have therefore developed concepts to describe on one side (1) the crop "carbon" economy or metabolic cost and on the other (2) the crop resource use efficiency.

Root lifespan and soil resource use

Every plant organs follow a cycle of birth, aging and death and their lifespan determines their resource uptake capacity over time. Therefore, the root system lifespan partly determines the soil resource acquisition and exploration strategy (Eissenstat and Yanai 1997) as well as the plant resource use efficiency (i.e. cost vs benefits) (Bloom et al. 1985). In general, species adapted to fertile habitats usually have a short root lifespan and high root turnover compared to species growing in soil with low fertility (Van Der Krift and Berendse 2002). Indeed, under high and homogeneous nutrient availability, having more profuse roots with a short lifespan would maximize soil resource exploration and use, see review by Chen and Brassard (2013). This strategy being advantageous as long as resource losses associated to root and shoot mortality are compensated by resource uptake. However, under natural conditions, soil nutrient pools are usually scattered and relatively variable in time and space. Therefore under such conditions, the short-term benefit of fast root proliferation and short root lifespan constitute a disadvantage over the long-term due to soil patches depletion (Fransen and Kroon 2001). Species with lower root proliferation and longer lifespan would be more appropriate to maximize soil resources use efficiency. In general, large diameter roots usually have a longer lifespan than finer roots and increase in temperature and in temperature variability tend to shorten root lifespan (Eissenstat and Yanai 1997; Van Der Krift and Berendse 2002).

Water availability is variable in time and space. Since water can be replenished from rainfall, having roots with a longer lifespan is beneficial, as long as the respirational cost of roots located in dry soil layers could be reduced (Eissenstat and Yanai 1997). In this regards, development of root cortical senescence or aerenchyma could be advantageous. Maintaining metabolically "cheap" roots with a long lifespan would allow for the uptake of water and nutrients after re-wetting of dry soil layers, likely from newly formed laterals. In general, addition of water to the soil increases root lifespan but saturated water conditions decrease it, due to stresses associated to anoxic conditions (McCormack and Guo 2014). In grapevine, it was shown that root lifespan in dry soil layers also depends on the level of hydraulic redistribution that occurs from the "wet" to the "dry" part of the root system which prevents roots from drying out (Bauerle et al. 2008). Tolerance to desiccation also depends on the root response *via* increased suberization in the exodermis and endodermis, which increases the root lifespan (Enstone et al. 2003). At location where soil water availability increases with depth, deep roots are expected to have a longer lifespan, see review by Chen and Brassard (2013). Along this line, a study from Goins and Russelle (1996) showed that deeper roots (i.e. 60 cm) of alfalfa had a longer lifespan than shallower ones. In contrast, shallow roots are expected to have a shorter lifespan that is more correlated to seasonal rainfall, as observed for trees (Maeght et al. 2015).

In most cropping systems, the lifespan of the aboveground biomass is determined by the plant phenology or the management practices (i.e. mowing, grazing or harvesting). Observation from a one-year experiment on grassland systems showed that aboveground biomass removal, either by herbivory or mechanically, stimulates root growth and turnover and overall increases the productivity of the system (Frank et al. 2002). This has also been observed in intermediate wheatgrass where increase in forage harvest was found to increase total root biomass and to decrease root lifespan during the first three years of growth (Pugliese et al. 2019). In alfalfa, root lifespan was shortened after the first harvest but not after the second, suggesting stronger tolerance to shoot removal and increase in root production over time (Goins and Russelle 1996). However, in the long-term root growth and lifespan of 5-years old alfalfa plants decreased due to aging and sensitivity to seasonal conditions (Pietola and Smucker 1995). Root lifespan of perennial crops is of particular interest as it is critical to the long-term advantages of root proliferation. Therefore, long term resource use efficiency of perennial cropping systems, which take into account establishment, relocation and senescence of plant organs, especially roots, requires more attention from the scientific community. However, studying root lifespan is particularly difficult under field conditions and little is known about root lifespan and turnover among crop species, depth and over long periods of time.

Rhizoeconomics

Crop production has many analogies to business economics. It uses resources (e.g. water, nutrients) that can be saved (i.e. stored) and transformed into products (i.e. seeds, leaves, stems) and this product can contribute to the acquisition of resources (Bloom et al. 1985). Therefore, crop biomass production could be split down to their metabolic costs with respectively a production and a maintenance cost. When focusing on roots, the metabolic cost of soil exploration, also called rhizoeconomics, plays a central role in determining plant strategy for capture and efficient use of resources (Lynch 2007b). It is generally associated with crop ideotype breeding (i.e. the selection of the ideal crop phenotype), which can only be efficient when specific root traits and associated performances are understood. In particular, root traits that reduced the metabolic cost of soil exploration were found particularly advantageous under limited water and nutrient availability by enabling greater root growth and resource uptake for a given metabolic investment (Lynch 2015). In doing so, two main strategies apply (1) to select cultivars with an optimal number of roots (i.e. reduce production costs) and (2) to select cultivars with a reduced root respiration rate (i.e. reduced maintenance costs). When focusing on water uptake, a clear example of strategy (1) comes from

Gao and Lynch (2016) who found that maize cultivars with reduced crown root number had deeper rooting, deeper water uptake and greater yield under drought. Similar results were obtained for maize cultivars with reduced lateral root branching (Zhan et al. 2015). Strategy (2) is also highly relevant because approximately one quarter to two third of the carbohydrates synthetized daily could be lost through root respiration, depending of the plant species and age, see review by Lambers et al. (2000). For example, maize cultivars with fewer cortical cell files or having larger cortical cells had a reduced respirational cost, deeper rooting and water uptake and greater yield under drought (Chimungu et al. 2014a, b). Reducing root respirational cost *via* aerenchyma formation or root cortical senescence has proven to enhance deeper root growth and nutrient use efficiency under low nitrogen and phosphorus availability in maize and barley (Saengwilai et al. 2014; Schneider et al. 2017). When focusing on deep resource use, all these results converge towards the "steep, cheap and deep" ideotype developed for maize, which is supposed to develop a deep root system advantageous for water and nitrogen capture (Lynch 2013). These latter performances being inferred from root traits that reduce the cost of subsoil exploration such as, crown roots with steep growth angle, fewer lateral roots and low cortical respirational cost.

Resource use efficiency

The term crop resource use efficiency has been utilized to characterize the capacity of crops to take up resources and to convert them into biomass or marketable products (i.e. different definitions apply). Because of the trade-off between plant nutritional supply and plant growth, crop resource use efficiency is defined either as an increase in crop productivity (i.e. maximize production per resource uptake) or an increase in crop resource use (i.e. maximize overall resource uptake) (Gourley et al. 1994; Blum 2009). Improving crop nutrient use efficiency is a challenge worldwide in both high- and low-input systems but won't be treated as part of this review. When focusing on water use, crop performance depends on the water availability and on how the plant partition the use of that water over the day and season. In particular, the flowering and grain filling stages are the most sensitive stages to water deficit for most crops (Allen and Pereira 1998). Water use efficiency is usually characterized by the amount of biomass or grain produced per mm of water evapotranspired. Maximum water use efficiency for grain production of wheat is estimated at 20 kg $ha^{-1}mm^{-1}$ over the season (French and Schultz 1984). Normally, water use efficiency increases after anthesis and during grain filling and could reach up to 55 kg ha^{-1} per extra mm of water transpired during those stages (Manschadi et al. 2006). In this latter study, greater transpiration use efficiency was identified for cultivars with a deeper root system and preferential access to deep stored water. Ultimately, deep water uptake that occurs late in the season, during sensitive stages, is highly valuable for grain production and was found to be three times more efficient than overall seasonal water efficiency (Kirkegaard et al. 2007). Transpiration efficiency (i.e. crop yield/water transpired) reflect the intrinsic plant water use efficiency whereas crop water use efficiency (i.e. crop yield/total evapotranspiration) reflect the water productivity at the crop or field level. Indeed, evaporation losses at the field level are important and practices such as mulching or growing species with fast leaf expansion could increase the water productivity (Molden et al. 2010). In the end, optimal water use, defined as a way that maximize soil water extraction while ensuring sufficient water for the flowering and grain filling period, should be seen as main goal for a given context (Passioura and Angus 2010). As mentioned earlier, crops could be divided in two categories based on their water usage/strategy, (1) crops with relatively low transpiration rate and a more water saving strategy and (2) crops with a high transpiration rate and a more water spending strategy. Under water limited conditions, growing crops with a water saving strategy was found advantageous to save water during the crop vegetative stages for later use in the season, during grain production (Passioura 1983; Lobet et al. 2014). Manipulation of wheat cultivars to make then more water saving was done by reducing the metaxylem vessel number in seminal roots which overall reduces the plant axial conductance (Richards and Passioura 1989). These cultivars have improved yield under drought but evidence of delayed soil water use was lacking. It is noteworthy to mentioned that crop selection for high water use efficiency under limited water supply has generally led to crops with reduced transpiration and productivity (Blum 2009). Therefore, when stored water is available at depth, focusing on effective use of water which aim at maximizing transpiration rather than water use efficiency, should be more advantageous. Following this approach, improved drought resistance comes from enhanced root growth at depth and maintained transpiration under drought, which promotes deep water uptake as found in studies on rice, coffee and wheat, respectively (Kobata 1996; Pinheiro et al. 2005; Kirkegaard et al. 2007). The majority of research on water and nitrogen efficiency have focused on resources utilisation efficiency because genotype differences in utilisation efficiency exist (Blair 1993; Blum 2009; Lynch 2015). However, differences in acquisition efficiency offers interesting perspectives. At the agricultural system level, water use efficiency also incorporates all inputs (e.g. rainfall, irrigation) and losses (e.g. leaching, evaporation) that could occur and will affect crop growth and the system efficiency, see section "G x E x M".

G x E x M

A major challenge when working on the optimization of agricultural systems, is that resource availability is highly variable in time and space and all potential deficiencies might occur synchronously. A clear example comes from the water-limited conditions of Australia where water was found not to be the sole limitations to crop yield (Fig. 19). In fact, yield differences could arise from (1) water availability, (2) nutrient availability, (3) crop and species grown, (4) farming context or (5) a combination of several factors. Crop resource use efficiency and productivity is therefore tightly dependent upon the genotypic characteristics of the crop (G), the environmental conditions (E) and the crop management (M). Proper understanding of theses G x E x M interactions that drive crop resource efficiency and productivity are of primary importance towards adapted agricultural development, especially in understanding the benefit of deep rooting to a certain farming context (Thorup-Kristensen and Kirkegaard 2016).

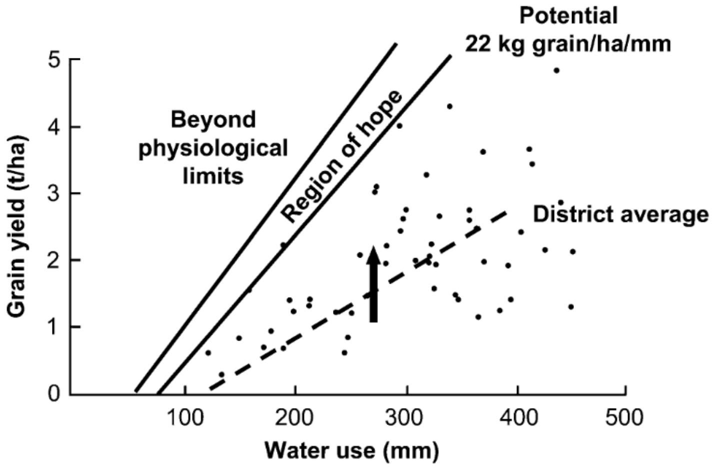
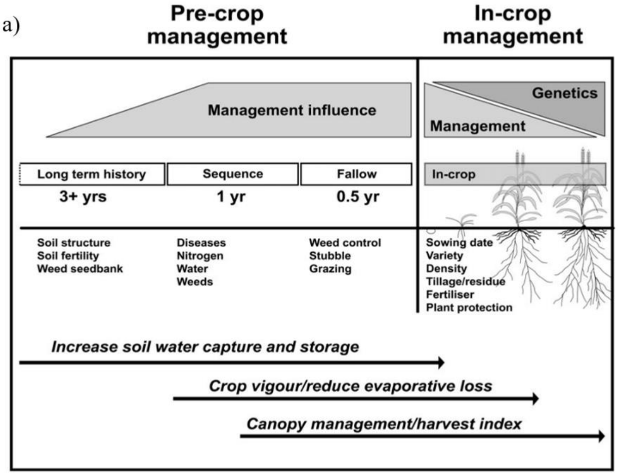


Figure 19. Wheat yield in relation to seasonal water supply. Australian districts performances (dot). The water limited potential being the potential yield obtained if only water was limited. Region of hope refers to possible genetic or management improvement. From (Kirkegaard and Hunt 2010)

A clear example of successful integration of G x E x M interactions in cropping practices comes from a pilot study carried out in Australia which aimed at improving water use efficiency of spring wheat (Kirkegaard et al. 2014). In this region, wheat cultivation occurs in mixed livestock-cropping systems and productivity is particularly constrained by the limited amount of rain that fall during the season (i.e. 350-550 mm of annual rainfall). When possible, a pre-crop (i.e. a fallow or legume pasture) is grown prior to cereals (in-crop) to build up the soil fertility and control weeds (Fig. 20a). In such dry conditions, the pre-crop cultivation could have important effect on water availability for the subsequent crop. The main challenge is therefore to maximize in-season water use and reduced off-season water losses (i.e. evaporation, fallow transpiration). A particular challenge is not to create any negative legacy effect over multiple years (i.e. overuse of stored water at once). A modelling study showed that it is favourable to grow a legume break crops when nutrient supply is limiting cereals yield (Lawes and Renton 2010). However, in regions where water constitutes the main yield limitation, controlling summer fallow weeds is advantageous to reduce plant cover and transpiration and increase the water stored in the soil (Hunt and Kirkegaard 2011). In fact, growing an alfalfa pre-crop would not allow subsoil water to refill in 76% of the seasons compared to annual crops (Lilley and Kirkegaard 2011). Further increase in soil moisture content could be accomplished by reducing livestock grazing to prevent evaporation by increasing stubble retention on the ground (Kirkegaard et al. 2014). All in all, these pre-crop management practices can increased water and nutrients stored in the soil allowing the cultivation of early sown crops with a slow maturing cycle. Early sown crops due to their extended growth and deeper root growth were found particularly advantageous and adoption of this practice was estimated to nationally increase yield by 0.5 t/ha (Hunt et al. 2019). Development of wheat cultivars with a a)



b)

Scenario	Management lever	Mean yield increase: all years (kg ha^{-1})		Mean yield increase: 2000–2009 (kg ha^{-1})	
		Additive	Singular	Additive	Singular
1	Baseline	–	–	–	–
2	Stubble retention	237 (15)	237 (15)	52 (13)	52 (13)
3	Fallow weed control	956 (61)	769 (49)	935 (224)	759 (182)
4	Crop sequence	647 (41)	158 (10)	507 (122)	47 (11)
5	Early sowing	563 (36)	467 (30)	324 (78)	222 (53)
6	New genotype	563 (36)	−154 (−10)	844 (203)	−36 (−9)

Figure 20. (a) Typical pre-crop and in-crop management practices found in southern Australian dry-land farming that influence the water use efficiency and productivity of cropping systems. (b) Simulated mean yield increased of each farming lever (singular) or in sequence with others levers (additive). The period 2000-2009, also called the millennium drought, corresponds to particularly dry conditions in the region. From (Kirkegaard and Hunt 2010)

particularly long coleoptile were developed to allow deeper sowing in wetter soil layers (Kirkegaard and Hunt 2010). The combination of these pre-crop and in-crop management factors favoured deeper water uptake and higher water use efficiency in this dry context. While individual practices effect on wheat yield are noticeable in comparison to the baseline (Fig. 20b), the best results were obtained when combining all those practices together highlighting the synergistic effect behind G x E x M interactions. However, this impressive pilot study reminds us that finding the best G x E x M synergy is highly dependent on the local conditions and practices. For example, adoption of break crops or early sowing could also lead to a yield penalty in some of the studied regions, in case of subsoil limitations for example (Lawes and Renton 2010; Hunt et al. 2019). In fact, when water stored in the subsoil is limited, having a relatively parsimonious root system was found to be advantageous and should be considered (Richards and Passioura 1981b, a).

Successful integration of G x E x M interactions on crop nutrient use efficiency is highly relevant but won't be treated extensively as part of this review. It noteworthy to briefly mention a study from Thorup-Kristensen and Kirkegaard (2016) who showed improved nitrogen use efficiency from growing deeper rooted crops that were more efficient at reducing subsoil nitrogen content. Overall they found that both crop choice and fertilisation rate had an influence on the amount of subsoil nitrogen and that such effect was carried over the three seasons of cultivation. Although these studies highlights that root traits such as deep rooting could be advantageous for multiple agricultural challenges (e.g. deep water and nitrogen capture), the value of specific root traits lies in understanding the farming context and the G x E x M interactions specific to it. Indeed having a shallow or parsimonious root system could be advantageous in some contexts, particularly for phosphorus acquisition or for saving water (Richards and Passioura 1981b; Burridge et al. 2019) Breeding for specific root phenotypes seems to offer promising perspectives by encompassing G x E interactions (Lynch 2019; Svane et al. 2019b). Nonetheless, identification of the fitness landscape of the phenotype and of associated management challenges should not be discarded. In some cases, benefits that can be gained from appropriate G x E x M synergy outweigh single genotypic benefit (Thorup-Kristensen and Kirkegaard 2016).

Annual vs perennial crops

Current food production systems are dominated by annual crops. Historically, the domestication of annual crops have always been predominant but perennial crops have often been considered and used (Meyer et al. 2012). Production systems that emerged from the green revolution focused on building highly productive varieties with reduced labour requirements to meet the increasing food demand. This lead to the cultivation of a limited number of annual crops and the generalisation of intensive practices (e.g. mono-cropping, tillage practices, chemical pest and weed management, etc.). When focusing on yield, technological progress and market values, annual crops are often more attractive in the short term but environmental consequences in the long-term have been neglected (e.g. environmental pollution, soil fertility losses, etc.). Many of the agricultural issues originate from the low plant diversity and the lack of perenniality in cropping systems. Recent approaches that aim at tackling these issues are usually separated into either approaches that focus on environmental/conservation aspects or production/social aspects. However, building productive and agro-diverse cropping systems seems an interesting long-term goal that considers both environmental and production aspects (Will, 2008). Along this line, agricultural practices that aim at increasing the number of species cultivated per land area, such as intercropping, mixing crop varieties and cover cropping have now proved to provide significant benefits for nutrient and water management in some agricultural context (Thorup-Kristensen and Rasmussen 2015; Fletcher et al. 2016; Wang et al. 2016). Increases in crop diversity was also found to mitigate effects of weather variability and improve yield stability over the long-term (McDaniel et al. 2014; Gaudin et al. 2015). With food productions systems facing the "perfect storm", more drastic cultivation approaches are required. The concept of perennial cropping systems emerged from the observation of natural ecosystems where productivity is maintained from a high level of nutrient cycling associated with a low level of nutrient loss, the most impressive example being the tropical rainforest (Field et al. 1998). Closer to agroecosystems, studies on perennial grassland reinforce the fact that productivity can be maintained over time without reduction in soil fertility (Jenkinson et al. 1994, 2004). In fact, perennial crops, with their drastically different life cycle offer an understudied set of crops that have interesting characteristics for the management of agricultural ecosystems (Pimentel et al. 2012).

Biomass allocation and yield

Apart from their life cycle, one of the main differences between perennial and annual plants is related to their resource allocation. In general, plants allocate resources to sustain two main functions: growth and reproduction. Therefore, from an evolutionary point of view there is a survival/fecundity trade-off associated to the plant resource allocation strategy (Bazzaz et al. 1987). Annual crops usually allocate a higher amount of resources to reproduction compared to perennial crops where more resources are allocated to survival organs (e.g. roots, leaves) (Bazzaz et al. 1987). These allocation patterns could be seen as a way to achieve long-term survival by tolerating stresses rather than achieving short-term reproductivity. But counter examples exist (Crews and Dehaan 2015). In addition, perennial crops usually have slower growth in their early development stage in comparison to annual crops, which may be due to their generally smaller seed size and longer life cycle (Ward et al. 2011). Nonetheless, over time perennial crops usually have a more profuse and extensive root system compared to annual crops, which is advantageous for water and nutrients uptake as well as for soil carbon sequestration (Tilman et al. 2002; de Oliveira et al. 2020). Furthermore, perennial crops allocate more biomass to aboveground organs other than seed compared to annual crops which constitutes an attractive advantage for biofuels and forage production. These latter differences are driven by differences in allocation strategy but also their longer crop cycle and higher light, water and carbon capture (de Oliveira et al. 2020). This difference in resource allocation (i.e. survival vs. reproductive) constitutes one of the major criticisms often made against the breeding and development of perennial grain crops. Little empirical data are available on perennial crop resources allocation and on potential gains that could arise from the selection of perennial grain crops. However, examples on fruit trees and wild perennial plants suggest that there is good prospects that selection for higher grain yield is achievable (Cox et al. 2006; Crews and Dehaan 2015). Maintaining or increasing grain yield over several years constitutes another challenge, as perennial crops have been found to reduce their grain production after one or two years (González-Paleo et al. 2016). Two breeding strategies are currently under development (1) interspecific hybridization (i.e. crossing an annual crop with its perennial relative) and (2) direct domestication from wild perennial crops, see review by Kantar et al. (2016). Breeding perennial grain crops is highly challenging and time consuming but initial results from the intermediate wheatgrass domestication program shows that increase in kernel weight is possible, albeit modest compared to annual crops (Dehaan et al. 2018). Ultimately, if grain yield of perennial grain crops stay lower than annual crops, the possibility of managing them as a dual-use crop (i.e. grain and forage) could still be attractive for farmers with livestock productions (Pugliese 2017).

Effect on soil properties and the environment

With their different life cycle, above and belowground biomass production and reduced soil management, perennial crops induce important modifications to the surrounding environment.

- *Physical properties*

Cultivation of perennial crops with a taproot system like alfalfa or lupine increases the soil biopores density and influence the root growth of subsequent crops by allowing growth of roots with a higher diameter (Han et al. 2015). Perennial crops because of their permanent ground cover reduce soil disturbances associated to soil management and soil compaction (e.g. tillage). In addition, fields with perennial crops are usually drier earlier in spring which increases the resistance to the weight of agricultural machinery (Marquardt et al. 2016). Perennial crops tend to improve soil structure and porosity which usually leads to greater water retention capacity, infiltration capacity, greater soil oxygenation, and increased root growth, which in turn facilitate water and nutrient acquisition, see review by (Kautz 2014).

- *Chemical properties*

Roots constitute one of the major sources of carbon deposition in the soil and growing crops with differences in root biomass allocation could have important influence on the carbon and nitrogen cycle (Crews 2005). Indeed, perennial grasslands have a greater amount of root biomass and soil organic matter in comparison to annual cropland (i.e. wheat) (Mikhailova and Post 2006; Glover et al. 2010a; Dupont et al. 2014). They also presented the highest nitrogen content when compared to relatively long-term fallow (Mikhailova et al. 2000). Perennial grassland, due to their extensive root system and water use reduce the amount of nitrogen that leached below the root zone. Perennial grassland were also found to be particularly efficient at reducing nitrogen fertilizer losses with up to 78% of nitrogen fertilizer being recovered in the shoots and roots after 18 years of cultivation (Jenkinson et al. 2004). However, this latter study showed that on a one year study both winter wheat and perennial grassland were as efficient at capturing nitrogen fertilizer with approximately 84% of the nitrogen applied being recovered in the shoots and roots. The increase in soil organic matter observed in perennial grassland and cropland is supposed to come from (1) reduced tillage and (2) higher biomass deposition belowground. Conventional tillage practices increase the mineralization of the organic matter and the leaching of nutrients, soil particles and chemical compounds into water stream (Seta et al. 1993). Even if the emergence of cover crops and no-till practices show a reduction in soil erosion and nutrient losses and tend to stabilize soil organic matter, it is unlikely that results from these practices will outcompete the performance of perennial cropping systems (Grandy and Robertson 2007). Indeed, perennial crops have already shown to significantly reduce nutrient leaching in comparison to annual crops (Randall and Mulla 2001; Jungers et al. 2019). Due to their higher and permanent ground cover, reduced soil disturbance and high water and nutrient use, perennial crops constitute an appropriate strategy to reduce soil erosion and nutrient leaching while building up the soil organic matter (Pimentel et al. 2012; Culman et al. 2013).

- *Biological properties*

By modifying the field environmental conditions (i.e. soil and plant communities), perennial cropping systems have a significant impact on biological communities. Higher insect communities such as herbivores, pollinators and detritivores are often found in perennial grassland likely due to the permanent ground cover and wider pollen diversity (Glover et al. 2010a). At the same location, Culman et al. (2010) found that higher levels of bacterial and nematode communities in the soil was correlated to the higher levels of soil organic carbon found in grassland in comparison to annual cropland. Dupont et al. (2014) also found that soil biological properties (i.e. mites and nematodes) are higher in perennial grasslands due to the presence of deep and numerous roots. Making analogy with no-till systems, reduced soil disturbance increases the density of beneficial organisms such as pollinators (e.g. ground-nesting bees) or seed-eating beetle, see review by Kantar et al. (2016). These observations suggest that perennial cropping systems present more complex biological communities in comparison to annual cropping systems due to the reduction in soil disturbance, continuous host and plant presence and improved soil fertility (Culman et al. 2010). At the landscape scale, perennial fields could also serve as bridges between nearby natural habitats and annual cropland and therefore increase the presence of beneficial organisms (e.g. biocontrol and pollinators) (Marquardt et al. 2016).

Extensive root system?

It is often mentioned that perennial crops are performing better due to their extensive root system. However, the author would like to remain cautious with such a statement as relevant data seem to be lacking. In the case of dicotyledonous species, such conclusion might be true as the tap-rooted system will grow gradually larger and deeper every year (Pietola and Smucker 1995). However, in the case of monocotyledonous species such as perennial cereals and grasses, it is still unclear how root continuity is maintained over time. If the seminal root system die off gradually and the nodal root system is disconnected from the whole plant after each tiller removal (Caradus and Evans 1977; Kirkegaard et al. 2015), then it is relevant to question which root system predominates after harvest, and especially at depth. If the majority of roots have to be re-established after each harvest therefore the efficiency of perennial cereals and grasses at exploiting soil and subsoil resources might be within the same range as winter crops. Following this reasoning, the uptake of resources from deep soil layers would also be limited after harvest. Our results support this hypothesis, as there is no change in soil moisture content at 0.75 m and 1.5 m depth after harvest under intermediate wheatgrass. In contrast, significant changes were observed for alfalfa, see chapter 4 "Deep water uptake of alfalfa *Medicago sativa* and intermediate wheatgrass *Thinopyrum intermedium*". Further research is needed but methodological limitations faced by root researchers echo to these concerns. To the author's knowledge, over- or underestimation of living roots of perennial crops can arise from (1) a too shallow sampling depth, (2) the difficulty to determine experimentally living from senescing or dead roots and (3) the fact that the presence of roots alone is not an appropriate indicator of root activity. Therefore the amount of roots sampled in perennial fields might reflect the overall root deposition at a time but not necessarily the amount of active root connected to the shoots. While it is certainly true that yearly root deposition is higher in perennial cropping systems, active amount of roots remain difficult to predict. In this regards, comparing the behavior of perennial grasses and grain crops after harvest in comparison to winter crops seems of particular interest. While the author do not attempt to minimize the fact that perennial crops usually present an extensive and deep root system that confers significant ecosystem services, understanding the advantages that such root system confers regarding soil resources uptake, biomass and grain production requires more attention and remain of primary importance for the development of perennial cropping systems.

Achieving long-term soil resource use and productivity while being sustainable?

The major differences between natural grassland and perennial cropping systems is the removal of nutrients during harvest. Therefore, the long-term effect of extensive use of soil resources by perennial crops on yield constitutes a key and complex goal in achieving sustainable and productive perennial cropping systems. Pugliese et al. (2019) showed that increased forage removal of intermediate wheatgrass increased the decline in soil inorganic nitrogen due to the greater above and belowground biomass production. While, some will be redistributed to the soil under the form of organic matter, such results still question the effect of long term depletion of soil nutrient stocks on the overall system performances. In addition, a study from the Rothamsted institute reminds us of the high year-to-year variability of grassland productivity likely driven by climatic conditions and nutrient cycling processes. Because long-term productivity of perennial cropping systems depends on maximizing soil resource use over multiple growing seasons, particular care should be paid to the depletion of soil nutrient pool, the nutrient and water cycle and carry-over effects from one season to the other. The environmental benefits and yield stability mentioned earlier will likely come when encompassing long period of time in comparison to annual cropping systems.

- *Perennial crop water use*

Perennial crops, like intermediate wheatgrass, due to their longer growing season and large transpiring biomass usually have a higher transpirational demand than annual crops (de Oliveira et al. 2020). In addition, perennial crops that have a deep rooted system have access to a reliable source of water that stabilize yield over time (Vico and Brunsell 2018). For example, alfalfa have been reported to extract water at soil depths greater than 4 m (Li and Huang 2008; Fan et al. 2016a). Differences in perennial crop root systems exist and shallow rooted perennial crops are more sensitive to water shortage (Monti and Zatta 2009). Here again a trade-off between water demand and water supply occurs as extensive use of water might lead to increased water stress over time as mentioned earlier. For example, a study by (Li and Huang 2008) showed that in an alfalfa/crop rotation, keeping the alfalfa phase longer than 8 years would induce strong water stress to the subsequent crop due to extensive water use at depth. Under conditions of water limitation, perennial crops with shallower roots and lower transpirational demand may be more appropriate.

- _Perennial crop nutrient use_

The effect of long-term perennial cropping on soil nutrient cycling is more complex as replenishment means of nutrients are more limited naturally, in comparison to water. In this regards, Crews (2005) established a framework with the aim to optimize nutrient uptake and limit losses over time. This framework is divided into three axes: (1) improving the synchrony between crop nutrient demand and nutrient supply, (2) targeting areas with soils of high weathering capacity and (3) replenishing the soil nitrogen content with legume crops. The first axes aims at optimizing nutrient inputs and crop demand in order to reduce the nitrogen losses that can be large in annual cropping system and cannot be replenished from the soil. There is room for improvement as nitrogen use efficiency of annual cereals is estimated globally to be around 33% (Raun and Johnson 1999)). Synchrony between demand and supply could be improved through increase in plant demand, precise nutrient management in time and quantity and higher nutrients capture (Fig. 21a), see review by Crews and Peoples (2005). The second axes focuses on soil characteristics that could ensure the supply of other nutrients (e.g. phosphorus and potassium) that could be

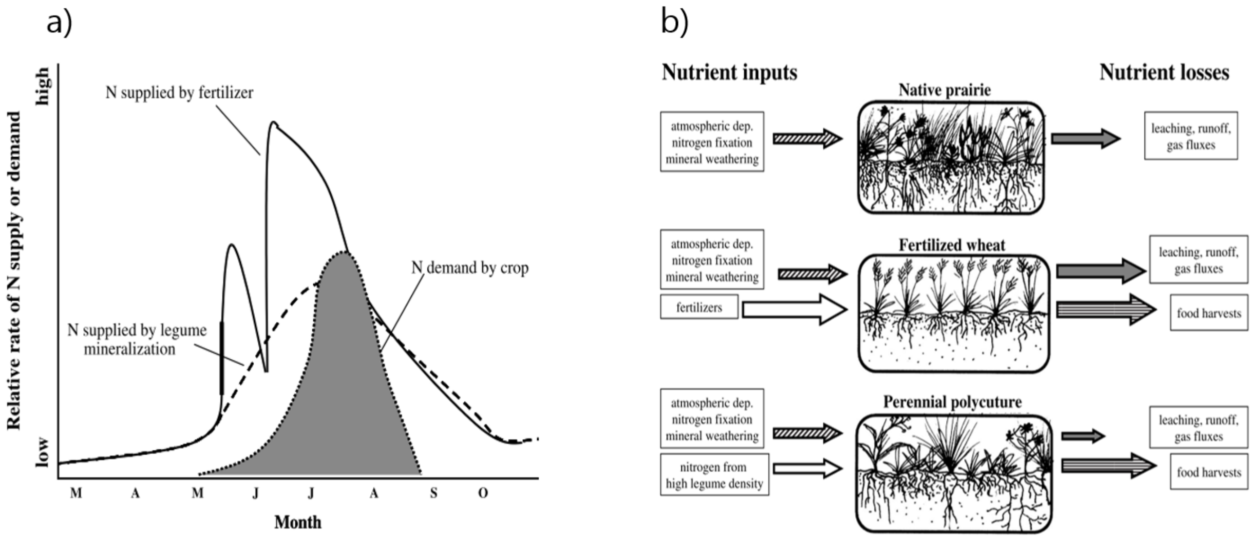


Figure 21. (a) Lack of synchronization in temperate annual cropping system between crop nitrogen demand and nitrogen inputs, whether under the form of a split fertilizer application or through mineralization of legume residues. (b) Nutrient inputs and losses in three different agro-ecosystems (i.e. native prairie, annual wheat and perennial polyculture). From (Crews 2005)

released *via* rock or soil weathering. This brings the question: can weathering of soil and rocks compensate the export from harvest? While the answer would be no when considering the harvest range of annual crops, learning from native grassland tell us that at locations with significant soil or rocks nutrient release substantial amount of yield could be achieved depending on the soil type, stage of development and climate, see review by Crews (2005). The third and final axes targets the incorporation of leguminous crops in the crop rotation, with the aim of reducing the use of inorganic nitrogen fertilizer application. This will also increase the system synchrony as release of nitrogen from legume residues is more closely related to crop demand (Fig. 21a) (Crews and Peoples 2005). However, nitrogen losses from organic origin could occur and should not be underestimated. There is room for developing perennial cropping systems with reduced external input of nutrients and high nutrient use efficiency while maintaining productivity over time. The efficiency of the system rely on the level of understanding of complex phenomena that occur within the soil (e.g. mineralization, soil weathering), the plant (e.g. nitrogen fixation, nutrient demand) and the air (e.g. atmospheric deposition, nitrous oxide release) (Fig. 21b). To date concrete examples outside natural ecosystems are lacking and more research should be done on finding the G x E x M interactions required for successful perennial cropping systems.

Toward more successional farming?

In ecology, succession defines the change and influence of biological communities on an ecosystem over time. Successional changes are often associated with the ability of an ecosystem to recover its natural functions following disturbances (e.g. fire). In agro-ecosystems, the natural succession of plant species is often disrupted by cropping decisions and tillage which induce changes that limit productivity and increase their negative environmental impact (e.g. nutrient leaching, soil organic matter decrease, weed pressure). Crews et al. (2016) defined highly disturbed systems (i.e. annual cropland) as in an early successional state and proposed a conceptual model for moving from such early successional state to a mid-successional state where lower level of disturbance is achieved by the integration of perennial crops in the rotation. From a production point of view, transitioning from mostly growing annual crops to totally perennial crops is unimaginable and not desirable today. Therefore, the challenge is to estimate the ratio of annual/perennial crop and the crop type succession that will maximize jointly the productivity and the ecosystem benefits. Agro-ecosystems in a mid-successional state are expected to have higher levels of soil organic matter due to reduction of tillage practices and higher belowground biomass deposition due to perennial crops. High deposition of soil organic matter will sequester carbon and nitrogen, but may reduce nitrogen availability early in the transition. In such a system, nitrogen fixing plants are of high importance to compensate the nitrogen being lost through harvest and sequestration. Table 1 shows a hypothetic crop sequence which aims at maximizing productivity while increasing soil organic matter, nutrient availability and reducing the risk of nitrogen losses. After each perennial phases, cover crops are grown to capture the nitrogen released that follow the perennial phase termination. Annual cereals are grown every three years to benefit from the improved soil fertility and maximize grain production. The first perennial phase focuses on reducing soil disturbance and grain production while the second perennial phase aims at rebuilding the soil nutrient pool. Depending on the location, focus on either nitrogen or phosphorus availability could be done *via* integration of crop with different root phenotype, greater nitrogen fixation or greater phosphorus mobilization, such as lupine (Davis 1991). Even if this system is speculative, grains could be produced in 5 seasons out of 7 or in all seasons depending on the crop choice of the second perennial phase. This sequence highlight the role that cover crops and advanced sowing technics still play in transitioning from early- to mid-successional systems. Three main questions remain: (1) how long should the perennial phase last in order to improve the soil fertility and ecosystem services? (2) how long should the annual cropping phase last in order to retain some of those benefits? and (3) how productive could such a system become in comparison to common annual grain production? For this latter question, breeding for productive perennial crops is of central importance. Such system might still be at a lower grain productivity than common annual cropping systems. However, if improved soil fertility, nutrient cycling and ecosystems services could be achieved over time and considering the high weather variability and the relative volatility of market price, such system is expected to be significantly more resilient and sustainable. More research on perennial crop breeding and long-term evidence of the benefit of such system are badly needed.

Year	**1**				**2**				**3**				**4**				**5**				**6**				**7**				**8**
Seasons	**A**	**W**	**S**	**S**	**A**	**W**	**S**	**S**	**A**	**W**	**S**	**S**	**A**	**W**	**S**	**S**	**A**	**W**	**S**	**S**	**A**	**W**	**S**	**S**	**A**	**W**	**S**	**S**	**A**
Annual cereal	**DS – Winter cereal** Residues / Grains *Reduce weed pressure*														**DS – Sp. Cereal** Grains												**DS – Sp. cereal** Grains		
Perennial crop	**US – Perennial grain crop** Residues / Forage / Grains *Reduce soil disturbance / Extensive nutrient capture*																	**DS – Perennial Legumes** Residues / Forage / Grains *Nitrogen fixator and/or P enhancer*											
Cover crop													**T/H** residues *Catch N*												**T/H** Residues *Catch N*				

*Table 1. Hypothetic 7-year crop sequence established with the aim to maximize productivity while increasing soil organic matter and nutrient availability and reduce the risk of nitrogen losses. The two perennial phase (blue and orange) could last longer if productivity could be sustained. **Bold and underlined = Soil management/Sowing technic - Crop type**; Normal text = Agricultural production; Italic text = Ecosystem services. Note: A: Autumn; W: Winter; S:Spring; S: Summer; DS: Direct Sowing; US: UnderSowing; T/H: Tillage or Herbicide. * Spring crop duration extend into the autumn for ease of reading*

Farmer's adoption and commercialization

Interestingly, from farmers' perspectives in Sweden, main benefits of growing perennial crops would be: improved soil quality, reduced nitrogen leaching and greater resistance to soil compaction (Marquardt et al. 2016). The same group of farmers also identified, greater presence of weeds (associated with low competitiveness at the beginning of their cycle), the risk of perennial crops becoming weeds and increase fungal pressure over time as their main concerns with such system. Surprisingly, low yields of perennial grain crops did not appear to be a major concern. However, it constitutes a more important adoption factor for farmers specialized in cereal production in comparison to farmers with livestock. Coming back to our example, intermediate wheatgrass present interesting nutritional composition in comparison to wheat (Becker et al. 1991) but lower level of gluten and lower dough forming capacity which might still limit market adoption (Kantar et al. 2016). While it is still early to think that perennial grain crops will replace annual relatives, they might have relatively interesting niche market value in the medium term. To date, the potential in terms of biofuel and fiber production of others perennial crops seems even more promising due to increasing demand (Fulton et al. 2015). While productivity, in terms of grains, may hardly match the performance of current farming systems, it is likely that increased concerns and drawbacks of conventional farming practices will result in an increase in the cost/benefit ratio in favor of more perennial cropping systems. In this regards, research on perennial cropping systems has an important role to play in developing incentive toward such agricultural systems. A number of studies are already showing interesting advantages that such systems could have. The task remains enormous and the biggest limiting factor may not be where one would think it is. As stated by Jacob Weiner (2017) *"the 'limiting factor' for the development of sustainable agricultural systems is not our scientific knowledge, but the political and economic structures within which agriculture is practiced. Plant ecology, the subject of this Journal, has much to contribute to the development of sustainable agricultural systems. But this knowledge will only be used if society has sustainability as one of its primary goals".*

Root and xylem anatomy varies with root length, root order, soil depth, and environment in two perennial crops.

Status

In preparation for submission to Plant and Soil.

Authors list

Corentin Clément[1&2], Hannah M. Schneider[2], Dorte Bodin Dresbøll[1], Jonathan P. Lynch[2], Kristian Thorup-Kristensen[1]

[1] *Department of Plant and Environmental Science, University of Copenhagen, 1871 Frederiksberg, Denmark*

[2] *Department of Plant Science, Pennsylvania State University, University Park, Pennsylvania 16802, USA ORCID 0000-0002-7265-9790*

Abstract

Deep roots (i.e. > 1 m depth) play an important role in supplying crops with water when the topsoil is dry. Root anatomy and therefore hydraulic conductance has important implications for the uptake of soil water, particularly water located deep in the soil profile. We hypothesize that root and xylem anatomy changes as a function of root type, order and length and the extent of these changes governs the water uptake capacity in deep soil layers. Using laser ablation tomography, we compared the cortical area, metaxylem vessel number, size and their estimated axial conductance of intermediate wheatgrass and alfalfa roots, two deep-rooted perennial crops. The deepest roots investigated were located at 2.25 m soil depth in the field and at 3.5 m depth in rhizoboxes. Anatomical differences were characterized along 1-m long individual roots, among root types and orders as well as between environmental conditions. Under field conditions, axial conductance decreased with soil depth in both crops. Axial conductance also decreased with depth along individual roots and was significantly different across root types, classes and growth conditions. Overall, alfalfa, with greater axial hydraulic conductance all along the soil profile is better suited for deep water uptake in comparison to intermediate wheatgrass. These results were associated with changes in metaxylem vessel anatomy which is a plastic phene in both crops. Detailed evaluation of deep water uptake in crops requires consideration of root system hydraulic architecture and their plastic response to heterogeneous growing conditions.

Introduction

Water is the greatest yield-limiting factor globally and water will increasingly constrain food production in the future (Kummu et al. 2016). Crop yield, from a water supply point of view, depends on the amount of available water and how crops partition the use of that water over the growing season (Passioura and Angus 2010). In contexts where water remains available at depth, crops with fast and intensive water uptake might be advantageous, whereas in water-limited environments, saving water during vegetative growth will ensure that water in the subsoil is not used too rapidly, but some will remain available later on in the season for seed production (Ahmed et al. 2018a). In both conditions, growing crops with deeper roots conferred increased water (Lilley and Kirkegaard 2011, 2016; Fort et al. 2017) and nutrient uptake (Thorup-Kristensen et al. 2012; Thorup-Kristensen and Rasmussen 2015), when resource pools are available at depth. In this regard, perennial crops are drawing more attention due to their extensive root systems, especially at depth, and to their longer growing period which confers numerous agronomical and environmental services (Glover et al. 2010b; Pimentel et al. 2012).

Deep roots, defined as roots growing below a soil depth of 1 m (Maeght et al. 2013), have to cope with a wide range of environmental conditions (i.e. soil compaction, hypoxia, suboptimal temperature) which may affect growth and function (Lynch and Wojciechowski 2015). In fact, plant rooting depth depends on local subsoil conditions, farming practices, and root architecture and anatomy (Lynch 2015; Lynch and Wojciechowski 2015; Thorup-Kristensen and Kirkegaard 2016). Root type and architecture determine the soil exploration in time and space and are therefore determinant for water and nutrient acquisition (Lynch 2007a; McCormack et al. 2015). Most monocotyledonous species like cereals, develop a fibrous root system with seminal roots emerging from the seeds and nodal roots emerging from shoot nodes, whereas dicotyledonous species develop a tap-root system with a central root and a crown of basal roots from which different order of lateral roots emerge (Lynch 1995). In this second group, the primary root tissues develop secondary growth leading to radial growth, senescence of the root cortex and the development of secondary metaxylem vessels (McCormack et al. 2015; Strock and Lynch 2020). Root anatomical and architectural phenes ("phene" is to phenotype as "gene" is to genotype) are intrinsically linked as they both require metabolic costs for establishment and maintenance (Lynch 2007b). In particular, root phenes that reduce the metabolic costs of subsoil exploration favor deep rooting and improve crop performance under drought and low nitrogen availability (Lynch 2018).

When considering water flow from the root to the shoot, hydraulic conductance is a critical parameter and is usually divided into a radial and an axial component (Ryan et al. 2016). Due to the composite structure of roots, radial hydraulic conductance is thus inversely related to (1) the length of the flow path along the cortical area (Bramley et al. 2009), (2) aquaporin activity (Steudle and Frensch 1996) and (3) the degree of maturation of the endodermis and exodermis (Zarebanadkouki et al. 2016). In contrast, axial hydraulic conductance depends on the maturation, number and size of metaxylem vessels, which vary across root type, order, and along root length, although empirical data are still lacking see review by Bramley *et al.* (2009) and Vadez (2014). Axial hydraulic conductance also varies among species, genotypes, and within a single plant due to the differentiation of metaxylem vessels that occurs during root development in monocotyledonous species and the production of secondary metaxylem vessels in dicotyledonous species (McCully 1995; Vercambre et al. 2002; Watt et al. 2008). Changes in number and size of metaxylem vessels can have important impacts on the water uptake capacity of the plant, as flow is proportional to the fourth power of the vessel radius, as described by the Hagen-Poiseuille law (Nobel 2009). Thus, having roots with large and numerous metaxylem vessels might be advantageous when water is abundant at depth throughout the season. In contrast, when plants rely on limited stored water in the soil, having a low axial conductance associated with reduced diameter in xylem vessel confers a more water conservative strategy (Richards and Passioura 1989; Prince et al. 2018).

A paradox remains as deep root water uptake, especially under drought, is not as straightforward as one would think and crops seems to fail at taking up all the available water at depth (Passioura 1991; Prechsl et al. 2015; Rasmussen et al. 2019). In fact, deep roots often constitute the bottleneck for water supply under dry conditions, when the topsoil is dry (Passioura 1983). This paradox suggests that either (1) deep water uptake is limited morphologically i.e. anatomically and/or architecturally or (2) that plants develop water use strategies that do not favour deep water uptake even when water demand is greatest. Among other possibilities, differences in axial conductance in the deepest parts of the root system is one of the proposed explanations for this inconsistency (Garrigues et al. 2006; Pierret et al. 2006). In fact, despite their important role, little is known about the structure and functions of deep roots (Pierret et al. 2016).

Alfalfa, *Medicago sativa,* is a perennial legume crop widely used for forage production. Intermediate wheatgrass, *Thinopyruum Intermedium*, is a perennial grass extensively used for cattle forage. With genetic similarity to wheat, it offers good prospects for breeding a perennial grain crop and breeding programs are now lead by the Land Institute, Kansas, USA which has trademarked it under the name (*Kernza*$^{©}$) (Dehaan et al. 2018). To study the root anatomical differences among root classes, root types, growth conditions and with soil depth, the two crops were grown under four different growth environments-in solution culture, mesocosms, rhizoboxes and the field.

The aims of this study were (1) to characterize anatomical differences by root type and root order of two deep-rooted perennial crops, (2) to characterize changes in root anatomy along the length of individual roots, (3) study the effect of the growing environment on root anatomy and (4) to characterize root anatomical variations associated with increasing soil depth. We hypothesize that (1) intermediate wheatgrass and alfalfa display differences in root anatomy that vary with root type, order, length, soil depth and growing environment and that (2) root anatomical changes with soil depths explain differences in water uptake from deep soil layers.

Material and Method

Plant material

Intermediate wheatgrass seeds from a 5th breeding cycle (Dehaan et al. 2018) were provided from The Land Institute, Kansas, USA and alfalfa seeds cv. Creno were bought from Hunsballe frø, Denmark.

Solution culture study

Experiments were conducted in a greenhouse at University Park, Pennsylvania, USA (40°489N, 77° 519W), under 12-h photoperiod day/night, 18°C day/22°C night, 40%–50% relative humidity, with maximum illumination of 1,200 µmol photons m^{-2} s^{-1}. Additional light was provided when necessary with 400-W metal-halide bulbs. On the 4th of February 2019, intermediate wheatgrass seeds were germinated on moist germination plates until the emergence of primary leaves. After leaf emergence (~15 day after sowing), seedlings were transferred to a hanging basket above each 33L solution culture tank filled with aerated, half-strength Hoagland solution (Supplemental Table 2) (Hoagland and Arnon 1950). The pH of the solution culture was maintained between 5.5 and 6 and the complete solution was replaced every two weeks. On the 19th of March 2019, roots were counted by root type (i.e. seminal, nodal and associated lateral roots for intermediate wheatgrass and the taproot, 1st, 2nd and 3rd order lateral roots for alfalfa (McCormack et al. 2015)) on 8 plants per species. For each root type, two axial root segments (2 cm in length) and its associated lateral roots were sampled 10 cm from the root tip. Root segments were stored at 4 ℃ in 70% (v/v) ethanol/water prior to laser ablation tomography.

Field and rhizobox study

- *Climate conditions*

The experiment was conducted at Copenhagen University’s experimental farm (55.66815°N, 12.30848°E). Climate at the study site is considered a marine west coast climate (Cfb according to the Köppen climate classification), with 629 mm of annual rainfall (46-year average value) almost equally distributed throughout the year with an average reference evapotranspiration of 594 mm. The growing season spans approximately from April to September with a relatively high evapotranspiration 505 mm for only 343 mm of rainfall, on average.

Field experiment

Intermediate wheatgrass and alfalfa were sown at 20 kg seeds/ha on the 11th of April and 9th of September 2016, respectively on three experimental plots of 100 m^2, and kept under rainfed conditions. Alfalfa was sown in strips (1.5 x 10m), 3 m apart, as part of an intercropping experiment with spring barley (2017) and intermediate wheatgrass (2018). The soil is a stagnic luvisol sandy loam with approximately 20% of clay below 25 cm and a soil bulk density of 1.75-1.8 g/cm^3 (Table 1). Plants received 100 kg N/ha of NPK fertilizer (21-7-3; NH4:NO3 = 1.28) in early spring, every year. At each research plot, three soil cores were sampled down to 2.25 m on the 15th of January 2018 using a sonic drilling (www.arkil.dk). Soil samples were collected at specific depths of 0.5, 1.15, 1.75 and 2.25 m to collect root segments.

Table 1. Soil horizons physical and chemical properties in the three growth systems studied. Average value. Texture classification follows Danish standard. (OM) Organic Matter, (N) Nitrogen content, (P) Phosphorus content, (K) Potassium content, (Mg) Magnesium content. *Topsoil was common in the mesocosm experiment

	Soil depth	Soil bulk density	Clay <2µm	Silt 2µm-200µm	Sand 200µm-2.0mm	OM	pH	N	P	K	Mg
	m	g/cm^3	%	%	%	%		%	mg/100g	mg/100g	mg/100g
	0-0.5	1.57	8.7	8.6	81.0	3.0	7.3	0.3	4.0	9.6	4.4
Rhizobox	0.5-2.0	1.61	17.6	4.2	77.9	0.3	7.9	<0.03	0.6	3.3	4.2
	2.0-4.0	1.71	16	8.7	75.2	<0.1	8.0	<0.03	0.4	3.0	3.9
Mesocosm*	0-0.3	1.30	6.6	5.0	86.7	1.7	6.8	0.10	3.1	11	3.9
- Loam	0.3-1.0	1.50	18.9	18.6	62.3	1.2	7.8	<0.03	0.4	4.1	3.4
- Sandy loam	0.3-1.0	1.50	12.5	12.4	74.3	0.3	7.9	0.07	0.8	7.1	8.2
	0-0.25	1.43	14.6	16.1	67.9	1.6	7.7	0.10	4.7	11.5	7.3
Field	0.25-0.75	1.74	20.0	14.6	64.8	0.7	7.8	0.05	1.1	8.3	8.4
	0.75-1.5	1.75	19.5	18.2	62.1	0.2	8.0	<0.03	0.6	6.6	7.4
	1.5-3.0	1.83	20.0	19.3	60.7	0.2	8.0	<0.03	0.4	6.9	8.6

Rhizobox experiment

An outdoor rhizobox facility consisting of 4 m deep rhizotrons with a respective growing area of 0.36 m^2 (Thorup-Kristensen et al. 2020b) was used to grow alfalfa and intermediate wheatgrass. The rhizoboxes were filled to a soil bulk density of 1.6 g/cm^3 with soil taken on a sandy loam luvisol with on average 16% of clay (Table 1). Alfalfa and intermediate wheatgrass were sown on the 5th and 18th of July 2016, respectively. The crops received 60, 360 and 727 mm of irrigation in 2016, 2017 and 2018, respectively, in addition to rainwater and 100 kg N/ha of NPK fertilizer (5-1-4) in spring, every year. On the 5th of September 2018, soil samples were collected in two rhizoboxes per crop, horizontally with a hand auger at 0.5, 1.1, 1.7, 2.3, 2.9 and 3.5 m depth to collect root samples.

Root washing

In the laboratory, roots samples were obtained by manually washing them free of soil for each soil sample collected. Every root segment down to ~ 0.5 mm in diameter was retrieved. Root segments were stored at 4 °C in 70% v/v ethanol/water. Four random roots were selected for laser ablation tomography, for each soil depth, crop and growing environment.

Mesocosm study

The experiment was conducted in the greenhouse at the experimental farm of University of Copenhagen (55.66815°N, 12.30848°E). Plants were grown in mesocosms consisting of PVC cylinders 1.1 m in height by 0.073 m in diameter with a sealed bottom with drainage holes. From 0 to 0.3 m soil depth, the growth medium consisted of a mix of a loam soil collected on a luvisol to which 10% of potting mix was added. Below that depth, two subsoils collected below 2 m soil depth on a stagnic luvisol, were used. The two subsoils differed in texture, one being a sandy loam containing 12% clay and the other being a loam containing 20% clay (Table 1). Intermediate wheatgrass and alfalfa were sown in nurseries on the 15th of May 2018 and transplanted on the 1st of June 2018. A single plant was transplanted directly in each mesocosm in three replications per soil type. The experiments were carried out with natural light ranging from 500 to 1,200 µmol photons m^{-2} s^{-1} of photosynthetically active radiation with a temperature of 27 °C day/18 °C night. Plants were kept well-watered all through the experiment and each mesocosm was fertilized at 100 kg N ha^{-1} (Min have 5-1-4) *via* the water. On the 18th of September 2018, the whole mesocosm was cut apart and the entire root system was washed out. For each plant, the three deepest roots (i.e. nodal roots for intermediate wheatgrass and 1st order lateral roots for alfalfa) were sampled intact and cut in 10 cm segments from the root base to the root tip. On each root segment, the axial[1] root and one associated lateral root were sampled and stored at 4 ℃ in 70% v/v ethanol/water prior to laser ablation tomography.

Root sample processing

All root segments were preserved using a Leica EM CPD300 critical point dryer (Leica Microsystems), except for root segments from the solution culture experiment, which were too fragile. The root segments were sectioned with laser ablation tomography (Hall et al. 2019; Strock et al. 2019). Root cross sections were imaged with a Sony A7rII camera with a 53 micro lens (MP-E 65mm). Root cross-section images were analyzed using the image analysis software ImageJ (Schneider et al. 2012) with the plugin ObjectJ (https://sils.fnwi.uva.nl/bcb/objectj/). Root cross-sectional area, stele area, cortical cell number and size, and count and area of individual metaxylem vessels were manually measured using the software.

---

[1] An axial root is defined here as a root of higher branching order when related to its lateral root. An axial root is thus the mother root of a lateral root.

Data and statistical analysis

To describe the root cortical area and its change during plant growth we calculated the root cortex ratio as the ratio of the root cortical area to the root cross-sectional area. For alfalfa low values of the root cortex ratio (< 25%) correspond to secondary growth. Metaxylem vessel diameters were calculated from the vessel surface area, assuming that the vessel has a perfect circular shape. Axial hydraulic conductance, hereafter referred to as axial conductance, of metaxylem vessels was theoretically estimated using the Hagen-Poiseuille equation (Nobel 2009). Theoretical axial root conductance ($m^3\ s^{-1}\ MPa^{-1}$) was calculated for each cross-sectional image using the modified Hagen-Poiseuille law (Strock, 2018). Axial hydraulic conductivity ($m\ s^{-1}\ MPa^{-1}$) was calculated by relating axial conductance to root cross-sectional area and is an indicator of the efficiency (i.e. cost/benefit) of a root to transport water. All statistical tests were performed using the *R* software using the Rstudio interface (version 1.1.383; *R* core team, 2018) using the "lme4, version 1.1-21", "multcomp, version 1.4-10", "ggplot2, version 3.1.0" and "emmeans, version 1.4.3.01" packages. We used linear mixed models to look for variations in root anatomical phenes across growing conditions and soil depth. Normality and homoscedasticity of the data were checked by examination of the residuals and Q-Qplot. When data did not fulfill these two assumptions, a log transformation was applied to normalize the data. Overall effect of distance from the root base or soil depth were tested by an F-test with root anatomical phenes taken as dependent variables and distance from the root base/soil depth as fixed effect. Differences between root type, order and soil type were tested using pairwise comparisons of means with Tukey-adjusted p-values. Differences between soil depths were tested using pairwise comparisons of means with non-adjusted p-values.

Results

Root architecture and root class anatomical characteristics

At 43 days after planting, the root system of intermediate wheatgrass had developed approximately 3 seminal and 7 nodal roots (Fig. 1). Seminal roots had a 2- to 3-fold reduced cross-sectional area ($p \leq 0.05$) and were more branched than nodal roots (Table 2). Nodal axial roots had on average 5-6 cortical cell files and greater cortical area compared to seminal axial roots which had on average 3-4 cell files ($p \leq 0.05$) (Table 2). No differences were observed in the cortical area and number of cortical cell files between lateral roots originating from seminal and nodal roots. No differences were present in cortical cells size *per se* between root type and order. The cortical area represented approximately 75% of the root cross-sectional area in all root types and orders. Seminal axial roots appeared to have 1-2 central metaxylem vessels and nodal axial roots had 6-7 peripheral metaxylem vessels distributed around the stele (Fig. 2). However, lateral roots originating from both seminal and nodal roots had 1 central metaxylem vessel on average.

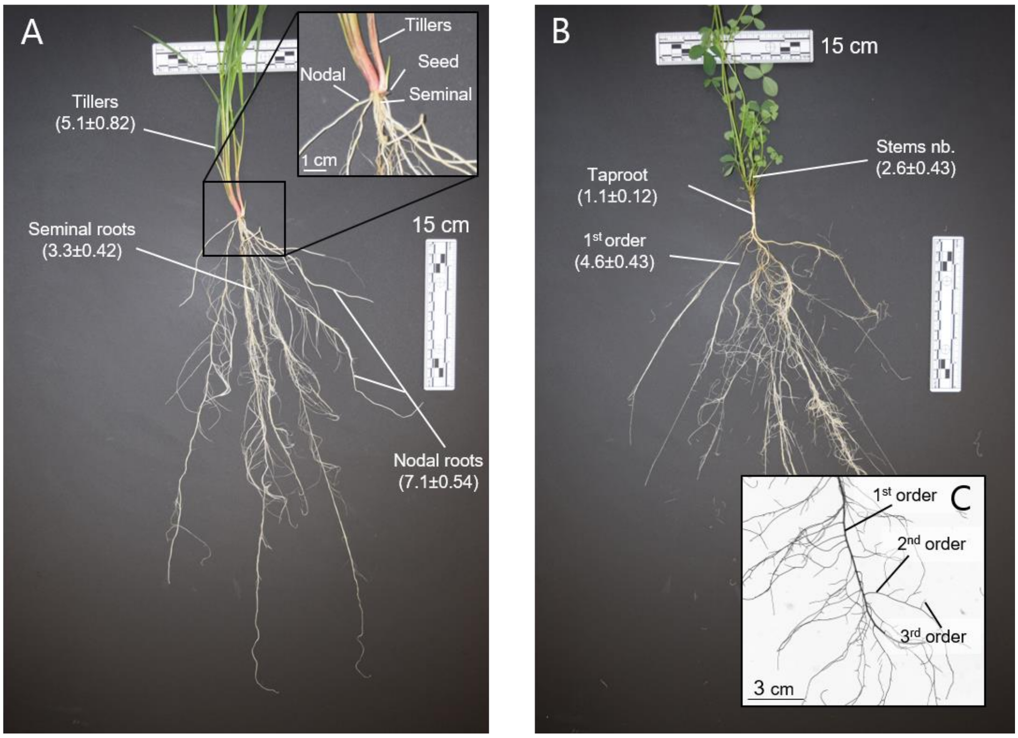


Figure 1. Root system architecture of intermediate wheatgrass (A) and alfalfa (B) of 47-d old plants. (C) Scanned 1st order lateral root of alfalfa. Nodal, seminal, taproot and four different order of lateral roots are indicated with average ± standard error over eight plants

Table 2. Comparisons of root anatomical phenes in seminal and nodal roots of intermediate wheatgrass and four different order of lateral roots in alfalfa grown in solution culture. Average values ± standard error. Comparisons were made between root classes and branching order, letters that differ indicate significant differences at (p≤0.05). Note: Cortical cells were not present due to secondary growth on the alfalfa roots sampled

	Intermediate wheatgrass				Alfalfa			
	Axial		Lateral					
	Seminal (n=16)	Nodal (n=16)	Seminal (n=16)	Nodal (n=15)	Tap-root (n=8)	1st order lateral (n=16)	2nd order lateral (n=16)	3rd order lateral (n=14)
Cross-sectional area (mm^2)	0.14±1.6e-2 (a)	0.40±3.7e-2 (b)	2.0e-2±2.3e-3 (a)	4.0e-2±6.6e-3 (b)	3.7±0.6 (a)	0.2±5.3 e-2 (b)	1.9e-2±6.8e-3 (c)	2.1e-3±1.5e-4 (d)
Cortex area (mm^2)	0.11±1.5e-2 (a)	0.30±3.2e-2 (b)	0.02±2.4e-3 (a)	0.03±6.0e-3 (a)	0.35±6.0e-2 (a)	0.04±7.4e-3 (b)	5.2e-3±1.5e-3 (b)	9.6 e-4±1.1e-4 (b)
Root cortex ratio (%)	74.9±4.2 (a)	78.8±1.3 (a)	74.5±5.5 (a)	73.3±4.5 (a)	11.5±3.5 (a)	22.3±3.2 (ab)	34.6±4.1 (bc)	46.5±4.4 (c)
Number cortical cell	3.5±0.5 (a)	5.6±0.5 (b)	2±0.3 (a)	2.3±0.4 (a)	NA	NA	NA	NA
Size of cortical cell 10^{-3} (mm^2)	0.9±1.1 (a)	1.1±0.1 (a)	0.4±0.04 (a)	0.6±0.1 (a)	NA	NA	NA	NA
Number metaxylem vessel	1.9±0.3 (a)	6.6±0.6 (b)	1.4±0.2 (a)	1.1±0.1 (a)	88.4±11.3 (a)	22.2±2.0 (b)	6.0±1.2 (c)	2.4±0.3 (d)
Metaxylem vessel diameter (µm)	5.5±0.1 (a)	5.2±0.1 (a)	3.3±0.1 (a)	3.9±0.2 (b)	5.3±0.1 (a)	4.3±0.1 (b)	3.1±0.2 (c)	2.4±0.1 (d)
Total conductance 10^{-7} ($m^3.MPa^{-1}.s^{-1}$)	0.2±0.04 (a)	0.4±0.04 (b)	0.003±0.001 (a)	0.01±0.01 (b)	7.8±1.3 (a)	0.7±0.2 (b)	0.03±0.01 (c)	2.8e-4±4.5e-5 (d)
Total conductivity 10^{-7} ($m.MPa^{-1}.s^{-1}$)	2.0±0.05 (a)	1.1±0.04 (a)	0.2±0.001 (a)	0.4±0.005 (a)	2.1±1.3 (a)	2.8±0.2 (a)	1.0±0.01 (b)	0.1±4.5e-5 (c)

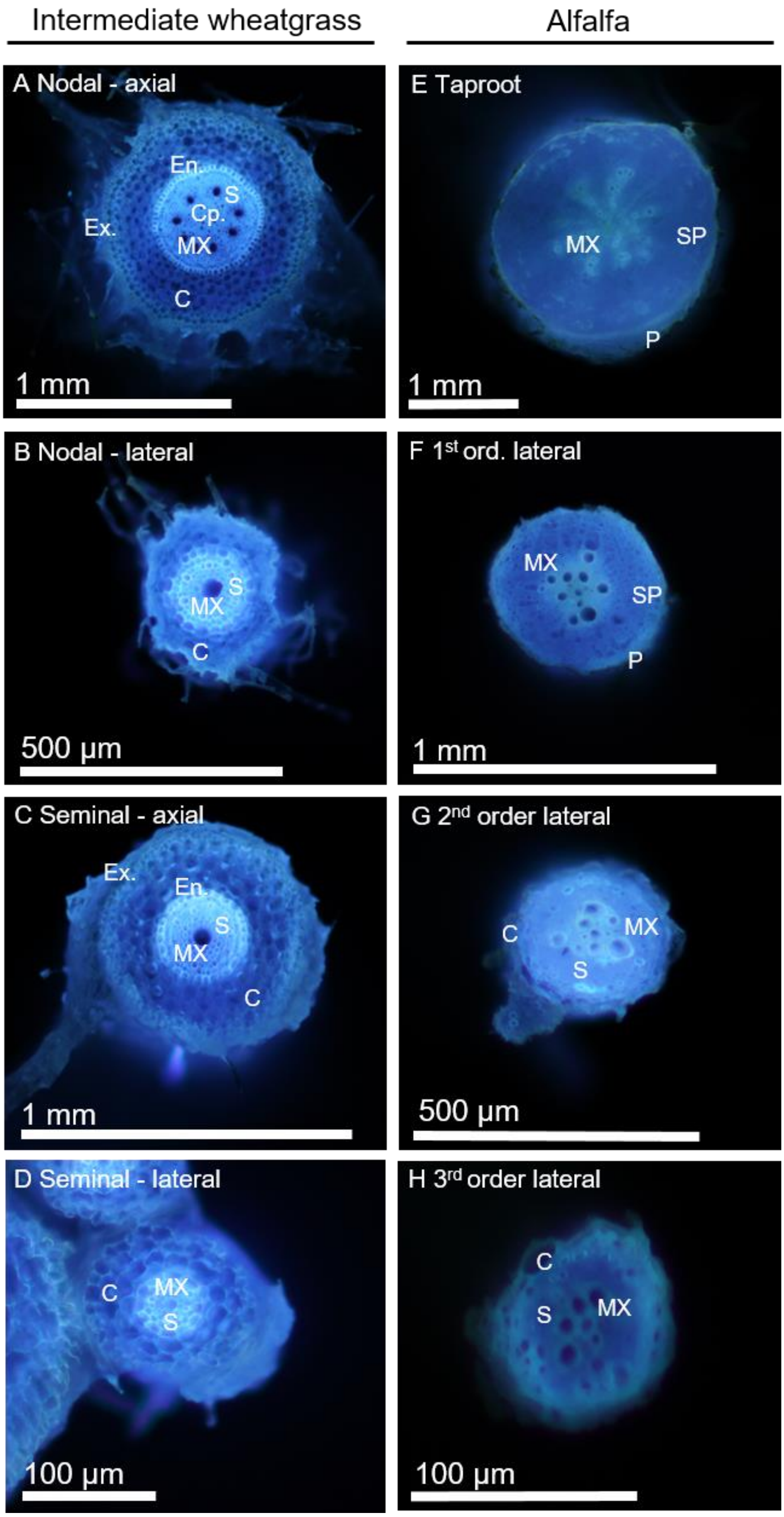


Figure 2. Cross-sections of seminal and nodal axial roots and respective lateral roots of intermediate wheatgrass (A-D; left panel). Cross-sections of alfalfa root, from the taproot to the 3th order lateral root (E-H; rigth panel). Annotations refers respectively to Central pith (Cp.), Exodermis (Ex.), Endodermis (End.), MetaXylem vessel (MX), Cortex area (C), Stele area (S), Periderm (P), Secondary Parenchyma (SP)

Metaxylem vessel diameter was reduced in lateral roots when compared to axial roots (Table 2). Lateral roots of nodal roots had significantly larger metaxylem vessel diameter than lateral seminal roots but no differences were found between seminal and nodal axial roots. With more numerous vessels and metaxylem vessels of larger diameter than seminal roots, both nodal axial and lateral roots appeared to have a 1.5 to 3-fold increased axial conductance, respectively. Alfalfa has a tap root system with a single taproot and approximately 5 1st order lateral roots at 43 day after planting (Fig. 1). Root cross-sectional area decreased as branching order increased ($p \leq 0.05$). Due to differences in rate of secondary thickening in the different root classes, cortical area and cortex presence increased along with increasing root order. Although we observed cortical cells that were collapsed, too few roots presented a complete intact cortical area which limits our conclusions regarding cortical cell number and size (Fig. 2). Number and size of metaxylem vessels and axial conductance decreased significantly with increasing root order. The taproot was 10-, 260- and more than 20000-fold more conductive than the 1st, 2nd and 3rd order lateral root, respectively. No differences were observed in total conductivity between the taproot and the first order lateral roots but total conductivity was 2- and 15-fold lower in 2nd and 3rd order lateral roots, respectively.

Root anatomical changes along individual root axes

- *Axial root*

For intermediate wheatgrass grown in mesocosms, root cross-sectional area and number of metaxylem vessels decreased from the basal to the distal part of the root (Fig. 3 & Fig. 4ac). Root cortical senescence was identified in nodal axial root as the root cortex disappeared preferentially in the basal part of the roots (i.e. 0-0.6 m) but no differences appeared between soil types (Fig. 4b). In distal parts of the roots, intermediate wheatgrass nodal axial roots had ~2 metaxylem vessels for both soil types, against ~8 and ~13 in basal parts of the roots (i.e. 0.1 m) when grown in a loamy and sandy loam soil, respectively (Fig. 4c). Indeed, significant differences between soil types in the number of metaxylem vessels were found in basal parts of the roots at 0.1, 0.2, 0.3, and 0.5 m, with a reduction of approximately 4-5 metaxylem vessels for roots grown on a loamy soil. Metaxylem vessel diameter increased between distances of 0.1 to 0.5 m and decreased from 0.5 to 1.0 m from the root base, with no differences identified between soil types (Fig. 4d). In the basal part of the root (i.e. 0 to 0.4 m), axial conductance increased in roots grown on the sandy loam and remained relatively constant in roots grown on the loamy soil (Fig. 4e). In the distal part of the root (i.e. 0.4 to 1.0 m), axial conductance decreased in both soil types as metaxylem vessel diameter and numbers decreased in both cases. Between 0.2 to 0.7 m from the root base, axial conductance was 1.5 to 3-fold greater for nodal axial roots grown on the sandy loam (Fig. 4e). Overall, axial conductance decreased by 3-fold and 8-fold between 0.1 to 1.0 m from the root base, when grown under a loamy or a sandy loam soil, respectively.

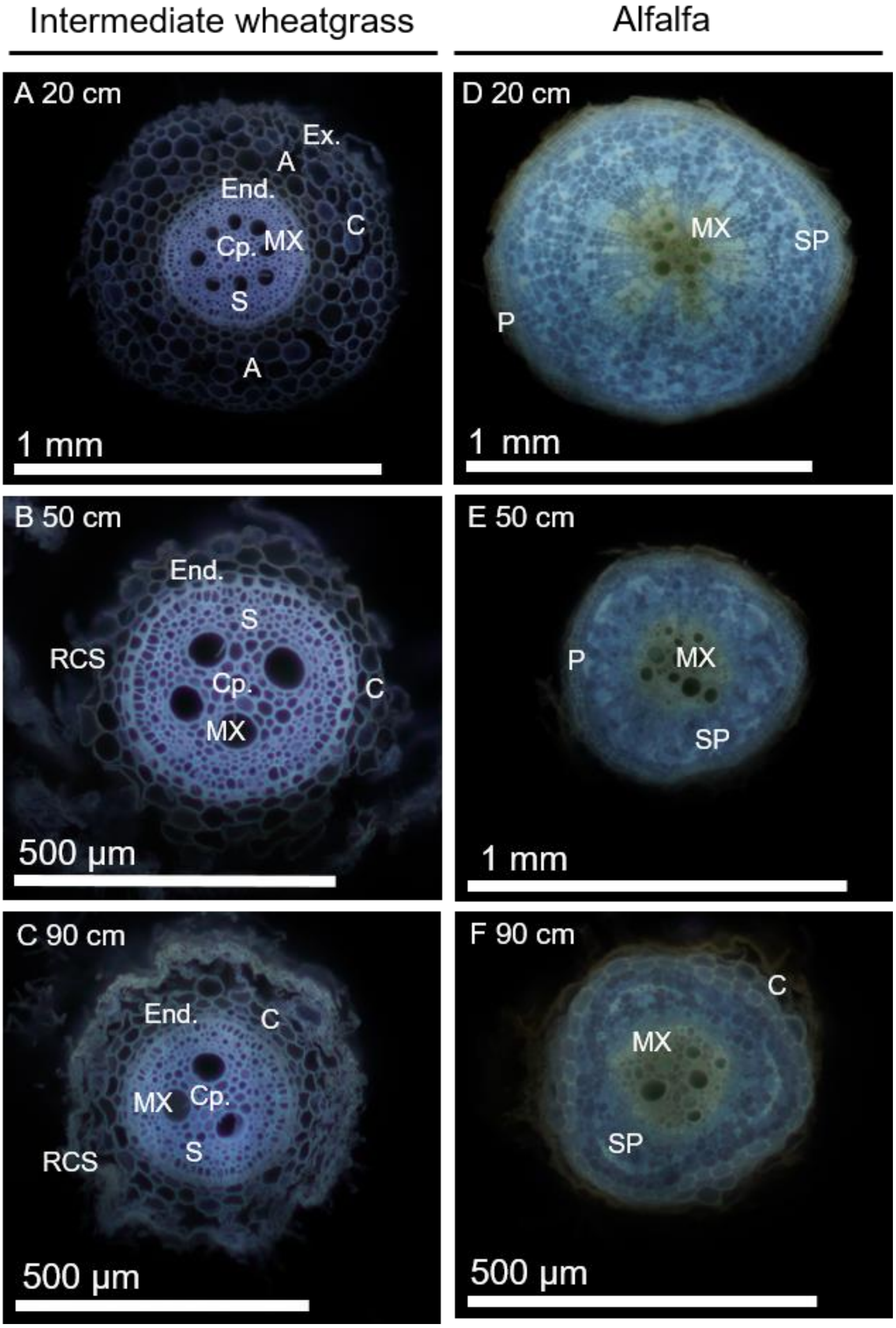


Figure 3. Consecutive cross section of intermediate wheatgrass (A-C; left panel) nodal axial root and alfalfa (D-F; right panel) axial root at 20, 50 and 90 cm from the soil surface at 107 days after sowing grown on a sandy loam. Annotations refers respectively to Aerenchyma (A), Central pith (Cp.), Exodermis (Ex.), Endodermis (End.), MetaXylem vessel (MX), Cortex area (C), Stele area (S), Periderm (P), Secondary Parenchyma (SP) and Root Cortical Senescence (RCS). Note: for photo (A) decision was made to present a root with little root cortical senescence and aerenchyma in the basal part to reflect the whole root anatomy of intermediate wheatgrass root

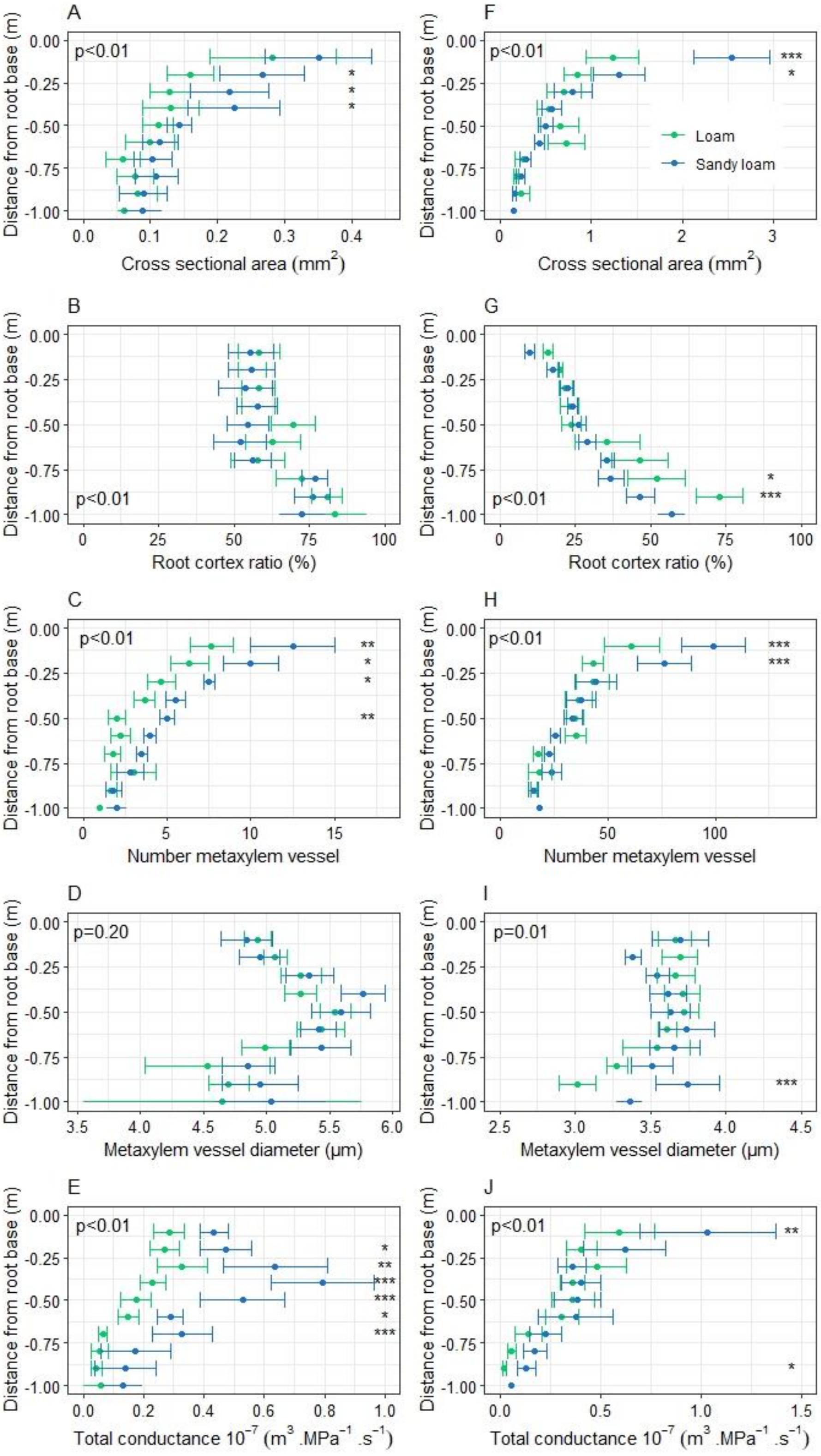


Figure 4. Evolution of root anatomical phenes along a 1-m long nodal axial root of intermediate wheatgrass (A-E; left panel) and 1-m axial root of alfalfa (F-J; right panel) grown in mesocosm on a sandy loam and a loamy soil, respectively. (A;F) Cross-sectional area, (B;G) root cortex ratio, (C-H) number of metaxylem vessel (D;I) metaxylem vessel diameter and (E;J) total conductance. Average value ± standard error. Comparisons were done at each soil depth between the two soil types. Asterisks indicate significance differences, respectively with * p≤0.05, ** p<0.01 and *** p<0.001. P values indicate results from F-test (Supplemental Table 1a)

For alfalfa, root cross-sectional area, number of metaxylem vessels, and axial conductance also tended to decrease from the basal to the distal part of the root (Fig. 3 & Fig. 4fhj). Differences in root cross-sectional area, number of metaxylem vessels, and axial conductance between soil types appeared only in the basal part of the root (i.e. 0.1-0.2 m). The root cortex was shed in the basal and older part of the root due to secondary growth (Fig. 4g). Cortical cells remained in the distal part of the root with significantly greater cortex presence under the loamy soil than under the sandy loam, respectively at 0.8 and 0.9 m from the root base ($p<0.01$). Between root length of 0.1 and 0.9 m, axial conductance decreased by 26-fold and 8-fold for roots grown on a loamy and sandy loam soil, respectively (Fig. 4j).

- *Lateral roots*

In lateral roots of intermediate wheatgrass (Supplemental Figure 1), we did not observe specific changes of root anatomical phenotypes along the root segment. Root cortical senescence did not occur and the cortical area accounted for the majority of the root cross-sectional area (~80-90%). Lateral roots had between 1 and 3 metaxylem vessels with a diameter of approximately 3.0 µm and had fewer and smaller metaxylem vessels when compared to axial roots. Metaxylem vessel diameter was variable and significantly larger in lateral roots originating at 0.4, 0.5 and 0.7 m from the axial root base when grown on the loamy soil. Axial conductance of lateral roots was approximately 50-fold lower than their parent roots. In lateral roots of alfalfa, we also did not observe specific changes of root anatomical phenotypes along the root segment. Secondary growth happened in lateral roots originating from the basal part of the parent root (i.e. 0.1 and 0.2m) as highlighted by the lower cortical area and greater number of metaxylem vessels. Further away from the root base, the cortical area of lateral roots comprised approximately 75% of the total root cross sectional area. Lateral roots of alfalfa had on average 5 metaxylem vessels with a diameter of 2.5 µm and had fewer and smaller metaxylem vessel when compared to axial roots. Axial conductance was 100-fold reduced in lateral roots compared to axial roots.

- *Crop comparison*

Overall, alfalfa had larger root cross-sectional area than intermediate wheatgrass and a greater number of metaxylem vessels in both axial and lateral roots. On average, metaxylem vessel diameter was smaller in alfalfa than intermediate wheatgrass, but axial conductance was greater in axial roots of alfalfa, especially in the basal part of the root (between root length of 0.1 and 0.5 m). In both crops, the axial root cortex appeared to be a temporary tissue due to root cortical senescence in intermediate wheatgrass and secondary growth in alfalfa. In lateral roots of both crops, cortical area represented more than 75% of the total root cross section and their axial conductance ranged between 0.05 to 0.5 10^{-9} $m^3.MPa^{-1}.s^{-1}$ This conclusion does not hold for lateral roots of alfalfa that originated close to the parent root base as these developed secondary tissues.

Root anatomy associated with soil depth

- *Field study*

In the field, intermediate wheatgrass root cross-sectional area and number of metaxylem vessels did not appear to differ significantly ($p=0.6$) with soil depth and were quite variable (Fig. 5ac). In contrast, metaxylem vessel diameter and axial conductance decreased gradually with soil depth ($p<0.01$) (Fig. 5de). Root cortical senescence preferentially developed in shallow soil strata, but was highly variable across the root profile with root cortical area representing on average 67% of root cross-sectional area (Fig. 5b & Table 3). Roots found at 1.75 and 2.25 m soil depth were 3- and 600-fold less conductive, respectively than roots in the topsoil (Fig. 5e). Two nodal axial roots were identified at 1.75 m, considering that the presence of a central pith and numerous metaxylem vessels are anatomical indications of nodal roots (Fig. 2 & Fig. 6b) as suggested by Watt, Magee and McCully (2008).

In the case of alfalfa, root cross-sectional area and axial conductance decreased gradually with soil depth ($p\leq0.05$) (Fig. 5ae). Metaxylem vessels number, to a lower extent, decreased with soil depth ($p=0.06$), with the greatest differences observed at 2.25 m (Fig. 5c). However, no differences appeared in metaxylem vessel size *per se*. Root cortical area represented on average 64% of the root cross-sectional area and no differences were found among soil depths (Fig. 5b). This suggest that we have sampled both roots with and without secondary growth. Deep roots at 1.75 and 2.25 m soil depth were 4- and 30-fold less conductive ($p\leq0.05$), respectively than topsoil roots. Alfalfa roots had a greater number of metaxylem vessels ranging between 18 and 6 vessels respectively at 0.5 and 2.25 m depth compared to 1 to 2 vessels per root in intermediate wheatgrass (Fig. 5c). Alfalfa roots had a greater axial conductance than intermediate wheatgrass, especially at depth (>1 m). On average, field grown alfalfa roots were 4-fold more conductive than intermediate wheatgrass roots (Table 3).

Table 3. Differences in root anatomical phenes of intermediate wheatgrass and alfalfa grown in the field and rhizoboxes. Average value ± standard error. Comparisons were made between growing environments, letters that differ indicate significant differences at ($p\leq0.05$)

	Intermediate wheatgrass		Alfalfa	
	Field	Rhizobox	Field	Rhizobox
Cross-sectional area (mm^2)	0.05±4.6 e-3 (a)	0.1±1.6e-2 (b)	0.2±3.0e-2 (a)	0.4±0.1 (a)
Root cortex ratio (%)	67.1±2.4(a)	59.8±3.2 (a)	64.3±2.4(a)	46.1±3.8 (b)
Number metaxylem vessel	1.9±0.2 (a)	2.6±0.3 (a)	13.9±1.4(a)	43.9±6.5 (b)
Metaxylem vessel diameter (µm)	4.5±0.2 (a)	5.0±0.2 (b)	3.8±0.1 (a)	3.6±0.1 (a)
Total conductance 10^{-7} ($m^3.MPa^{-1}.s^{-1}$)	0.2±0.1 (a)	0.5±0.1 (b)	0.9±0.2 (a)	6.0±1.7 (b)

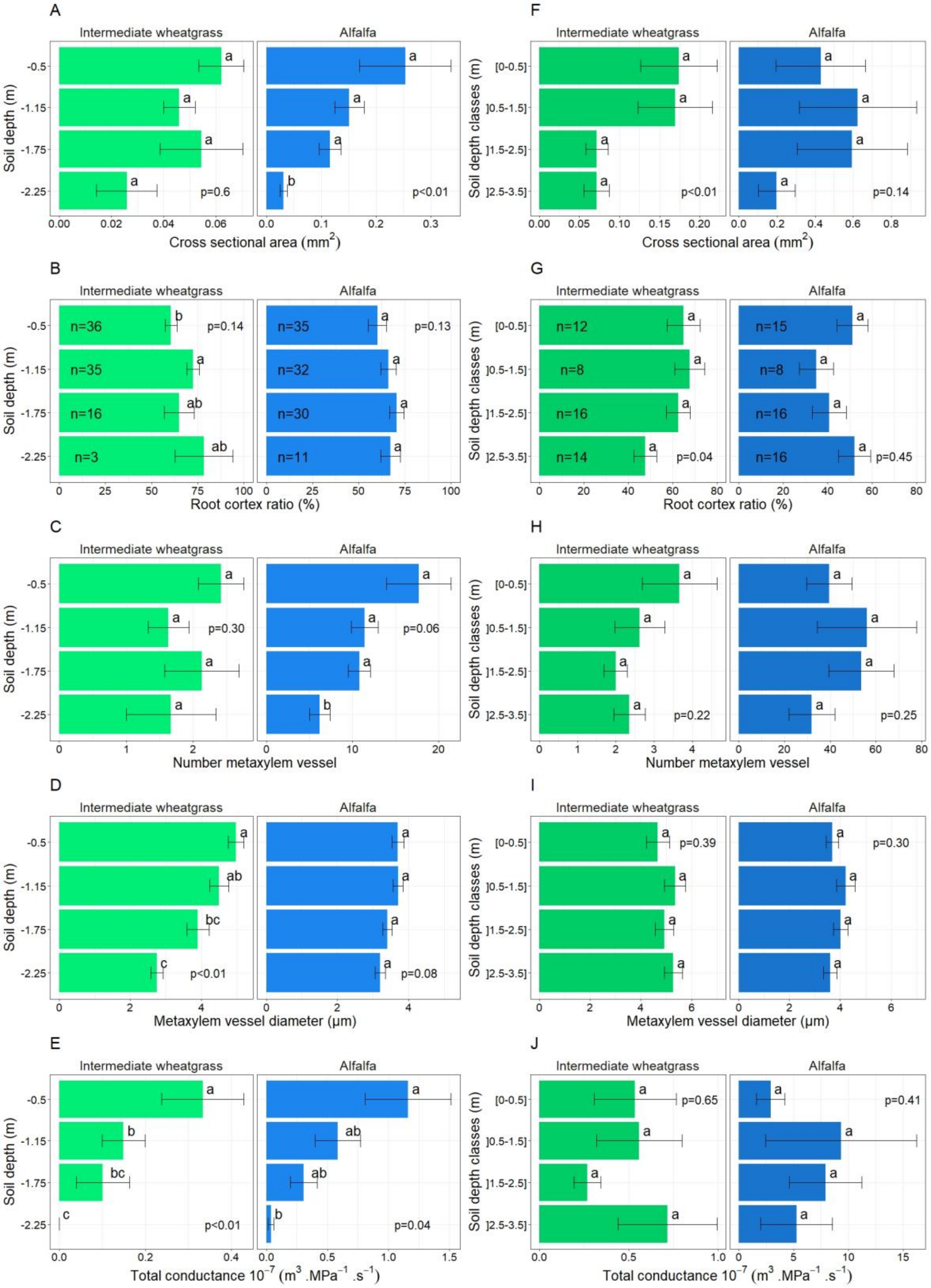


Figure 5. Influence of soil depth on root anatomical phenes of intermediate wheatgrass (green) and alfalfa (blue) grown in the field (A-E) and in the rhizoboxes (F-J). (A;F) Cross-sectional area, (B;G) root cortex ratio, (C;H) number of metaxylem vessel (D;I) metaxylem vessel diameter and (E;J) total conductance. Average value ± standard error. Comparisons were made between soil depth, letters that differ indicate significant differences at ($p \leq 0.05$). Note that field samples were collected at specific depth whereas data from the rhizoboxes are merged into soil depth classes for ease of interpretation. P values indicate results from F-test (Supplemental Table 1b)

- *Rhizobox study*

In the rhizoboxes, root cross-sectional area of intermediate wheatgrass decreased with soil depth ($p<0.01$) (Fig. 5f). The root cortex ratio, number and diameter of metaxylem vessels, and axial conductance were highly variable and within a similar range across all soil depths (Fig. 5ghij). In addition, axial conductance was greater at all soil layers compared to the field results. Nodal axial roots of intermediate wheatgrass were found at the bottom of the rhizoboxes (i.e. 3.5 m depth) (Fig. 6a). For alfalfa, no significant changes and differences with soil depths were observed for any of the anatomical phenes studied (Fig. 5fghij). As for intermediate wheatgrass, axial conductance was greater in all soil layers compared to the field results. Similar to field conditions, alfalfa was more than 10-fold more conductive than intermediate wheatgrass when grown in rhizoboxes (table 3).

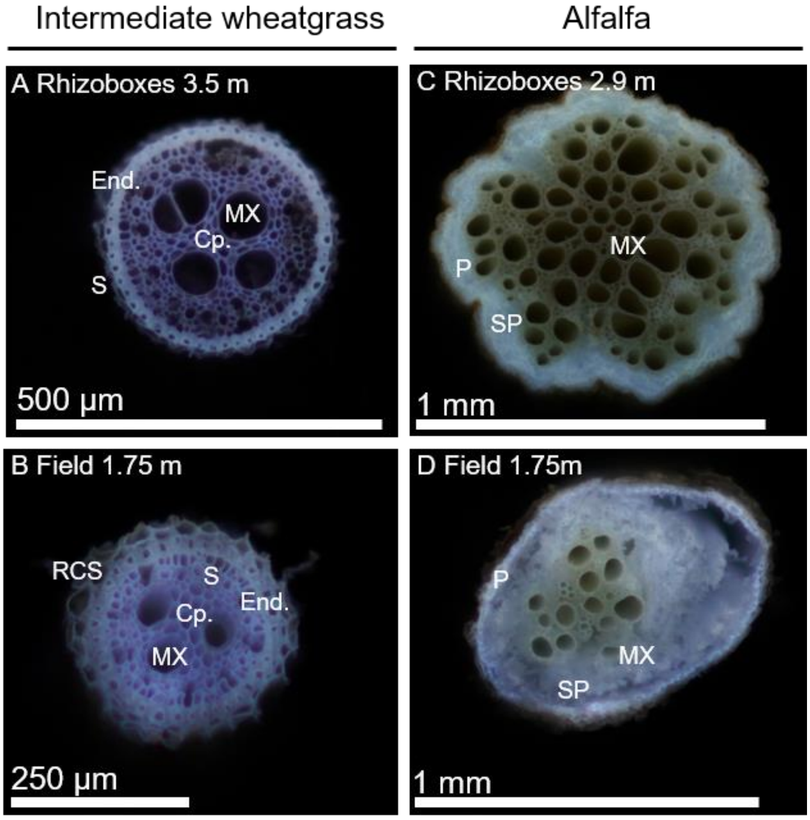


Figure 6. Cross section of intermediate wheatgrass (A;B; left panel) and alfalfa (C;D; right panel) roots growing at 1.75m and 3.5m soil depth in the field and rhizoboxes, respectively. Annotations refer respectively to Central pith (Cp.), Exodermis (Ex.), Endodermis (End.), MetaXylem vessel (MX), Cortex area (C), Stele area (S), Periderm (P), Secondary Parenchyma (SP) and (RCS) Root Cortical Senescence

Influence of the growth environment on root anatomy

For both crops, root anatomical phenes were expressed differently in the rhizoboxes when compared with the field (Fig. 5 & Fig. 6). In the rhizoboxes, roots of intermediate wheatgrass had on average twice the cross-sectional area of field-grown roots (Table 3). Root cortex ratio was approximately 10% lower in roots grown in rhizoboxes compared to the field. Overall, roots grown in rhizoboxes had larger metaxylem vessels and on average a 2.5-fold greater axial conductance ($p\leq0.05$). In the case of alfalfa, roots grown in the rhizoboxes had also a 2-fold bigger cross-sectional area and had a 20% reduced cortical area ($p\leq0.05$) (Table 3). Number of metaxylem vessels was 3-fold greater than roots grown in the field ($p\leq0.05$) but vessel diameters were similar. Axial conductance was on average to be 6-fold greater in the rhizoboxes compared to the field ($p\leq0.05$).

Discussion

To date, this is one of the few studies looking at root anatomy of agricultural crops growing below 1 m soil depth under field conditions. In both crops, hydraulic conductance decreased along individual root segments and with soil depth. Root phenotypes, including xylem and cortical phenes, were highly variable across growth environments and provide key insight into the crops subsoil exploration and deep water uptake capacity.

Root class and root type anatomy

We observed large differences in root anatomy between root type and root order. Seminal axial roots of intermediate wheatgrass expressed fewer metaxylem vessels than nodal axial roots as found for other cereals (Luxová 1989; Watt et al. 2008; Knipfer and Fricke 2011). Nodal axial roots presented a central pith, while this was not the case for their associated lateral roots. Nodal axial and lateral roots were larger in size and more conductive than seminal axial and lateral roots. However, seminal axial roots tend to have a greater specific conductivity than nodal axial roots as previously observed on barley (Knipfer and Fricke 2011). Nodal axial roots had a greater metabolic cost than seminal axial roots due to a greater cortical area to establish and sustain and therefore a greater demand of carbon and nutrient resources see review by Lynch (2015, 2018). The fact that lateral roots of seminal and nodal roots had fewer and smaller cortical cells suggests that radial water diffusion is less impeded across lateral roots compare to axial roots and reinforce the idea that they constitutes the primal organ of water uptake, as found for maize (Ahmed et al. 2016b).

In alfalfa, the taproot had the greatest hydraulic conductance, which highlights its role of collecting water from lateral roots and transporting it to the shoots. While the taproot and 1st order lateral roots were equally efficient at conducting water, efficiency decreased gradually in the 2nd and 3rd order lateral roots. Secondary root growth occurs in all four root orders, resulting in the sloughing of the cortex and secondary metaxylem vessel development (Simonds 1932). In fact, the taproot, 1st and 2nd order lateral roots with little cortical area had already transitioned from an absorptive to a transport function leaving the 3rd order lateral as the only roots involved in water uptake (see review by Mccormack *et al.* (2015)). This agrees with previous results showing that most of the water uptake occurs in lateral roots (Zarebanadkouki et al. 2013; Schneider et al. 2017).

In this study, we estimated root axial conductance using the Hagen-Poiseuille equation. This calculation assumes ideal capillarity in the vessel and overlooks the effect of intravascular pits, vessel maturity, length and degree of inter-connection (i.e. end walls structure), which can significantly reduce axial conductance (Vercambre et al. 2002; Orians et al. 2005). These metaxylem vessel parameters would require further attention as observation from 3D reconstructed images showed noticeable differences in vessel inter-connection between the two species

(Supplemental Figure 2). Intermediate wheatgrass roots presented continuous conduits (i.e. xylem vessels) aligned end to end whereas alfalfa root presented tracheids which are not aligned and discontinuous.

Anatomy changes along individual root axes

- *Axial roots*

Studying root anatomical changes along 1-m length of individual soil grown roots allowed us to observe that, for both species, root cross-sectional area, metaxylem number, and axial conductance tended to decrease from the basal to distal parts of the root. For intermediate wheatgrass, the increase in axial conductance toward the basal part of the root is supposed to be associated with the differentiation and the delayed development of metaxylem vessels in the distal part of the root (Frensch and Steudle 1989; Luxová 1989; Ahmed et al. 2016b). However, in the basal part of roots of intermediate wheatgrass such increase in axial conductance toward the root base does not hold as we observed a decrease or constant axial conductance, for roots grown in the sandy loam or loam soil, respectively. This was caused by a compensation of the reduction in metaxylem vessels number by an increase in vessels diameter. Such anatomical compensation was also observed in barley (Luxová 1989) and wheat (Kadam et al. 2015) exposed to water deficit. In alfalfa roots, the decrease in metaxylem vessel number from the basal to the distal part of the root was due to secondary growth, as for others woody species (Vercambre et al. 2002). In our case, changes in axial conductance were correlated with changes in metaxylem number *per se* and not size of the vessel. Decrease in axial conductance from the basal to the distal part of roots due to metaxylem development and maturation was also predicted in lupin (Zarebanadkouki et al. 2016).

- *Lateral roots*

We did not find any correlation between the anatomical phenotypes of lateral roots and their associated axial roots. However, similar anatomical variation and increase in axial conductance from the distal to the basal part of lateral roots have been previously reported (Luxová 1990; Zarebanadkouki et al. 2013, 2016). Root cortical senescence did not occur in lateral roots of intermediate wheatgrass and secondary growth in alfalfa occurred only in lateral roots that originated close to the axial root base, that are most likely older. This suggests that lateral roots of both species maintained a high radial conductivity compared to axial roots and that lateral roots constitute the main location of root water uptake (Ahmed et al. 2016b; Zarebanadkouki et al. 2016). Axial conductance of lateral roots were similar for both crops, whereas major differences in axial conductivity appeared in the axial roots. This support the idea that water flow capacity is limited by the hydraulic property of the axial/parent root as found by Pierret, Doussan and Pagès (2006).

- *Implication for water uptake*

For both species, cortical area increased from the basal to the distal part of the root due to root cortical senescence or secondary thickening, two processes known to significantly reduce radial hydraulic conductivity (Gambetta et al. 2013; Schneider and Lynch 2017). Root cortical senescence is usually correlated with increase suberization of the endodermis, as observed in intermediate wheatgrass (Fig. 2 & Fig. 3) but quantification of this process was out of the scope of this study. Nonetheless, this process considerably reduce the radial conductance, see review by (Enstone et al. 2003). Reduced radial conductivity associated with increased axial hydraulic conductivity in the basal part of the root is expected to favor water uptake from the distal part of the root (Zarebanadkouki et al. 2016). Thus both crops tend to adopt strategies that would favor water uptake from the most distal part of their root system over time. However, the decrease in axial conductivity in the distal part of the root might still limit the water uptake by these distal root segments, especially due to tension losses along metaxylem vessels (Ahmed et al. 2016b). In fact, reduced axial conductance in the distal part of the root and lack of sealing mechanisms (i.e. suberin lamellae, casparian bands) in basal part of the root system tend to "hydraulically isolate" the distal portion of roots (Zwieniecki et al. 2003). Thus, the decrease in axial conductance toward the root tip and the distribution of radial hydraulic resistance of axial roots are important factors that could favor deeper water uptake (Landsberg and Fowkes 1978; Zwieniecki et al. 2003). Such a conclusion does not hold for roots of alfalfa as radial sealing of the root will occur along with secondary growth. In this case, radial hydraulic conductance in lateral-absorptive roots and the low axial conductance in the distal part of the root seem to be the most important limitation to root water uptake in deep soil layers.

- *Influence of soil type*

In the mesocosm experiment, nodal axial roots of intermediate wheatgrass displayed a high degree of plasticity in response to the two soil types. However, we did not observe such plasticity in lateral roots. Main plastic responses were an increase in root cross-sectional area, metaxylem vessel number, and axial conductance, when grown in the sandy loam. Knowing that we have supplied optimal level of water and nutrients and that soil bulk density was similar and low, the most plausible reasons behind such plastic responses might come from differences in mechanical strength, water availability, and soil-root hydraulic resistances induced by textural differences (Jin et al. 2013). The sandy loam with less clay and silt might have had greater hydraulic conductivity, drainage capacity and less water holding capacity that could have induced lower water availability and harder soil layers. With greater cross sectional area and axial conductivity, roots grown in the sandy loam soil would allow greater penetration and water uptake from distal part of the roots. Increase in metaxylem vessels number as a response to drought have been previously observed in nodal roots of wheat (Kadam et al. 2015). Another explanation could be that due to the lower soil hydraulic conductivity in the loamy soil, roots reduced their axial conductance accordingly to keep the rhizosphere moist all the time and avoid the creation of air gaps and drop in water potential at the root surface (Carminati et al. 2013). Axial roots of alfalfa also responded differently to the two soil types. In the basal part of the roots, root cross-sectional area were larger in size and had more metaxylem vessels when grown on the sandy loam soil. In the same soil type, cortex presence was reduced close to the root tip and metaxylem diameter was significantly larger, which overall suggests a greater secondary growth rate in the sandy loam soil.

Root anatomy associated with soil depth

- *Root anatomy and axial conductance*

In the field and rhizoboxes experiments, soil coring enabled us to identify trends of anatomical variation with soil depth. However, it does not allow for the distinction of root type, order or tissue age which restricts the integration of associated functional effects (McCully 1999; McCormack et al. 2015). In intermediate wheatgrass roots, root cortical senescence occurs preferentially in shallow soil domains. This could be seen as a strategy to favour deeper rooting and water uptake by reducing the metabolic cost and radial conductance of the basal part of the root system (Zwieniecki et al. 2003; Lynch et al. 2014). Water absorbed by deep roots must travel a longer path and, assuming constant conductance, such path length would affect the crop water uptake by increasing the resistance to water flow (McElrone et al. 2004). In the field, the decrease in axial conductance with soil depth on the other hand suggest an increase in resistance to water flow with depth. As for annual cereals, the deepest roots had the lowest axial conductance for both crops (Watt et al. 2008). However, alfalfa roots had greater axial conductance than intermediate wheatgrass all along the soil profile. This is in line with results from water uptake measurements obtained in the field, which show deeper water uptake of alfalfa compared to intermediate wheatgrass (unpublished data, chapter 4).

In the rhizoboxes, axial conductance was variable but relatively constant across soil depth, which suggests a lower resistance to water flow especially in deep roots. Both crops had a reduced root cortex ratio which suggest greater root cortical senescence and secondary growth rate and reduced radial conductance. This could be interpreted as a strategy to increase deep water uptake *via* the sealing of the top root system (Zwieniecki et al. 2003). This is in line with root growth and water uptake measurements, which showed deeper root growth and water uptake for both crops when grown in the rhizoboxes than in the field (unpublished data). With greater axial conductance and root length density at depth in both growing environments, alfalfa absorbed more water from deeper soil layers than intermediate wheatgrass (unpublished data, chapter 4). In both growth environments, having access to a deep and reliable source of water was not correlated to specific root anatomical changes, as observed in trees (Pate et al. 1995; McElrone et al. 2004; Wang et al. 2015).

- *Influence of the growth environment*

Differences in root anatomical phenotypes between the two environments suggest a strong genotype x environment response. Both crops were deeper rooted compared to the field when grown in the rhizoboxes (unpublished data). Roots grown in rhizoboxes were larger, had a reduced root cortex ratio, had more numerous and larger metaxylem vessels and greater axial conductance than roots grown in the field. In addition, no differences were observed between these anatomical phenes and soil depth. In the rhizoboxes, groundwater or standing water is absent, soil temperatures are higher in the spring/summer and soil bulk density is lower than in the field. Evapotranspiration rate is greater due to increased wind speed associated to their elevated position, and increased soil temperature. Furthermore, because single plants are grown in rhizoboxes, the water demand per plant is greater than in the field, where plants are grown in denser stands. Improved root growth at depth is certainly associated to the capacity of intermediate wheatgrass to reduce the metabolic cost of soil exploration *via* root cortical senescence. Potentially explaining the presence of nodal roots all the way down to 3.5 m. While the influence of the greater water demand on deep root growth is certain, understanding the driver behind such response would require further investigation. When grown in the rhizoboxes, roots of intermediate wheatgrass had greater number and larger metaxylem vessels that would maintain hydraulic conductivity down to deep layers of the soil. Interestingly, differences observed between the loamy and the sandy loam soil in the mesocosm experiment were in the same order of differences as observed between the field and the rhizobox grown roots with a loamy and sandy loam soil type, respectively.

In the case of alfalfa, warm temperatures accelerate secondary growth (Simonds 1932; Tonn and Greb 2017) while waterlogging conditions induce cessation of secondary growth (Zook et al. 1986). These seems to be two plausible factors behind the enhanced secondary growth observed in rhizoboxes in comparison to the field as reflected by the large amount of metaxylem vessels observed in roots grown in the rhizoboxes (Fig. 6cd). Interestingly, metaxylem vessel size in alfalfa did not vary across environments and soil depth suggesting that alfalfa hydraulic plasticity is regulated by changes in secondary growth rate and metaxylem vessel number rather than diameter. Nonetheless, the greater axial hydraulic conductivity observed in the rhizoboxes, enable the plant to maintain hydraulic continuity over a longer path and to deeper soil layers.

Overall, none of these factors explain the lack of correlation observed between any root anatomical phenes and soil depth in the rhizoboxes but rather highlight the physiological plasticity and capacity of these two perennial crops to adapt to their environment in order to exploit deep subsoil layers. Root anatomical plasticity in response to soil compaction (Lipiec et al. 2012), drought

(Kadam et al. 2015) and temperature (Kaspar and Bland 1992) is common. While, such plasticity is beneficial in allowing exploitation of deep soil domains such results emphasize the need to recognize phenotypic variation across growth environments (Poorter et al. 2016).

Conclusion

Quantifying the hydraulic architecture of deep-rooted crops is challenging and empirical measurements are limited. This study illustrates the anatomical variations that occurs within a single root, either driven genotypically or by the environment. Both crops were well suited for deep water uptake (i.e. > 1 m). For both crops, axial conductance decreased along individual root segment and with soil depth in the field. Alfalfa with its taproot system ensures a high axial conductance and which may be an adaptation for enhanced water uptake from deep soil layers. Intermediate wheatgrass, with its fibrous root system had overall a lower root axial conductivity than alfalfa. Thus, intermediate wheatgrass plants might be restricted in their capacity to exploit deep water even if root cortical senescence might favour deep root growth and subsoil water uptake by reducing the metabolic cost associated to soil exploration and the radial conductivity in the basal part of the root system. However, seminal and nodal roots, with clear differences in metabolic cost and conductance might confer the plant a great potential and plasticity in environments with variable rainfall patterns but stored water in the soil.

Additional remark

Root cross-sectional area was a relatively good predictor of axial conductance (Fig. 7). The nature of the relationship between root cross-sectional area and axial conductance was quite variable among the different growth conditions. Nutrient solution data gave the best correlation in both crops. In the other environments, root cross-sectional area predicted appropriately the axial conductance for alfalfa but the correlation was weaker for intermediate wheatgrass.

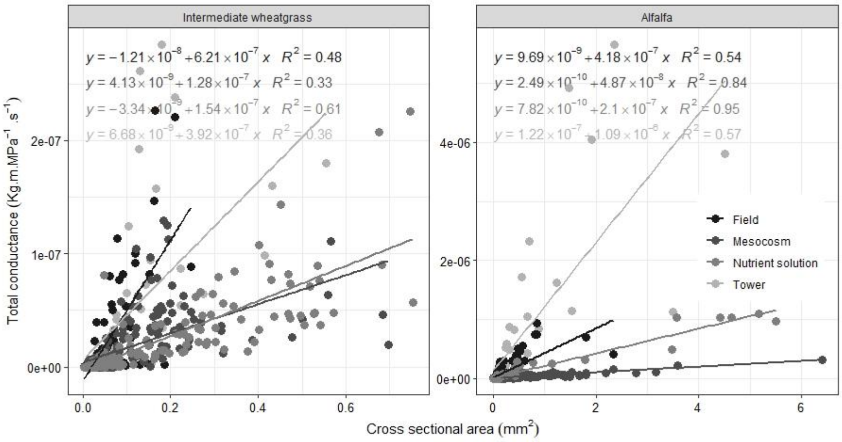


Figure 7. Relationship between the root cross sectional area and axial hydraulic conductance in different growing environment

Supplementary material

Supplementary tables

Supplemental Table 1a. Allometric analysis of the influence of soil depth on root anatomical phenes in the mesocosm with axial and lateral root distinction of intermediate wheatgrass and alfalfa

	Axial						Lateral					
	Intermediate wheatgrass			Alfalfa			Intermediate wheatgrass			Alfalfa		
	Int.	α	Int.	α	p	Int.	α	p	Int.	α	p	Int.
Cross-sectional area	-1.39	-0.02	**<0.01**	0.51	-0.03	**<0.01**	-3.71	0.0008	0.71	-2.95	0.001	0.69
Root cortex ratio	50.07	0.23	**<0.01**	5.85	0.50	**<0.01**	82.51	0.04	0.35	70.28	0.27	**<0.01**
Number metaxylem vessel	2.29	-0.02	**<0.01**	4.27	-0.02	**<0.01**	0.14	0.003	0.13	1.86	-0.003	0.10
Metaxylem vessel diameter	1.66	-0.0005	0.20	1.32	-0.0009	**0.01**	1.18	-0.001	0.13	1.08	-0.002	**<0.01**
Tot Conductance	-16.50	-0.03	**<0.01**	-16.23	-0.03	**<0.01**	-22.68	-0.006	0.31	-21.42	-0.02	**<0.01**

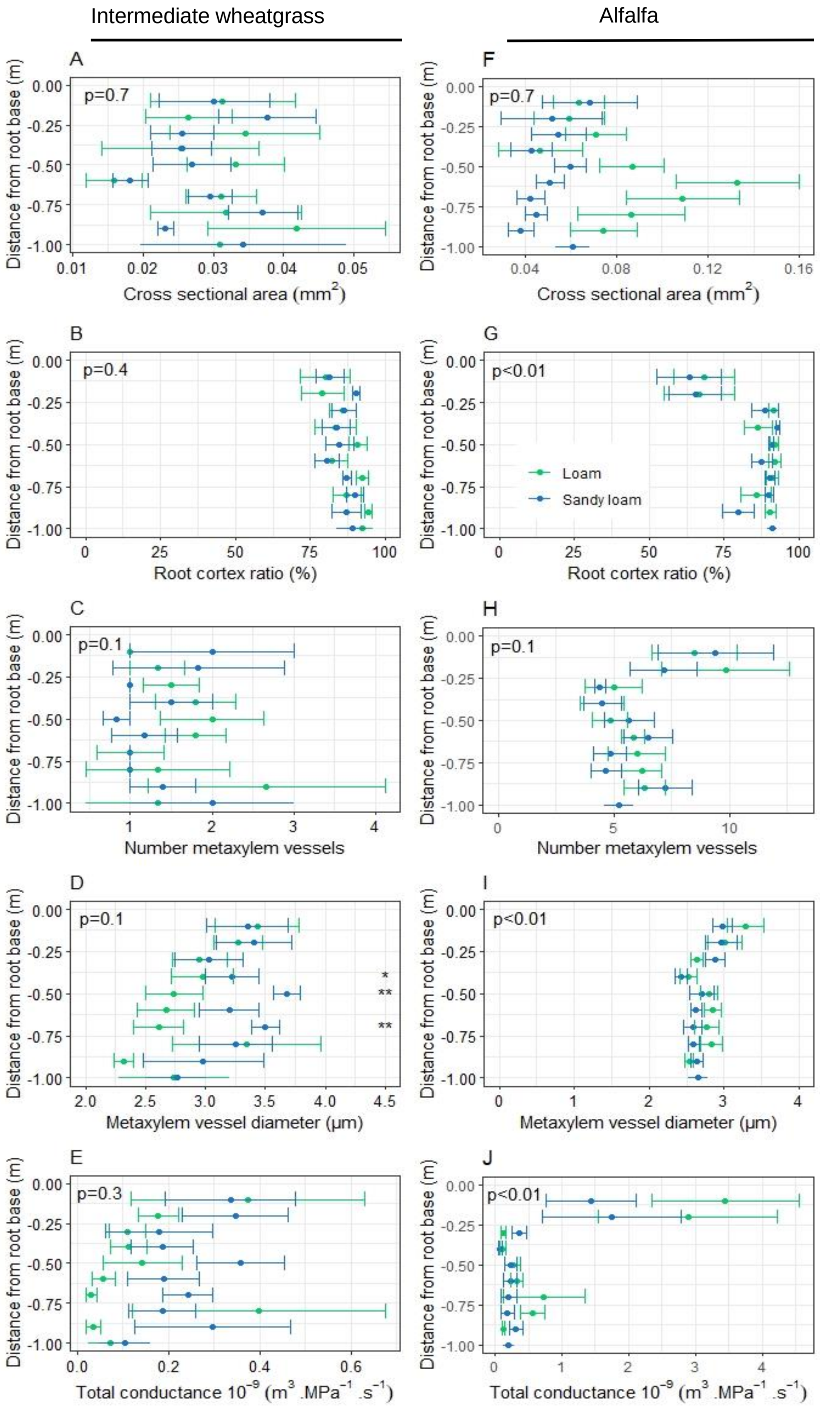
Supplemental Table 1b. Allometric analysis of the influence of soil depth on root anatomical phenes in the field, rhizoboxes of intermediate wheatgrass and alfalfa

	Field						Rhizoboxes					
	Intermediate wheatgrass			Alfalfa			Intermediate wheatgrass			Alfalfa		
	Int.	α	p	Int.	α	p	Int.	α	p	Int.	α	p
Cross-sectional area	-2.94	-0.0033	0.06	-1.94	-0.0045	**<0.01**	-2.05	-0.0034	**<0.01**	-1.68	-0.0028	0.14
Root cortex ratio	59.19	0.0706	0.14	58.10	0.0607	0.13	70.59	-0.0601	**0.04**	41.63	0.0252	0.45
Number metaxylem vessel	0.58	-0.0014	0.30	2.47	-0.0025	0.06	0.90	-0.0011	0.22	3.43	-0.0017	0.25
Metaxylem vessel diameter	1.69	-0.0023	**<0.01**	1.33	-0.0007	0.08	1.50	0.0004	0.39	1.37	-0.0003	0.30
Tot Conductance	-18.10	-0.0202	**<0.01**	-18.20	-0.0092	**0.04**	-19.31	0.0018	0.65	-17.03	-0.0035	0.41

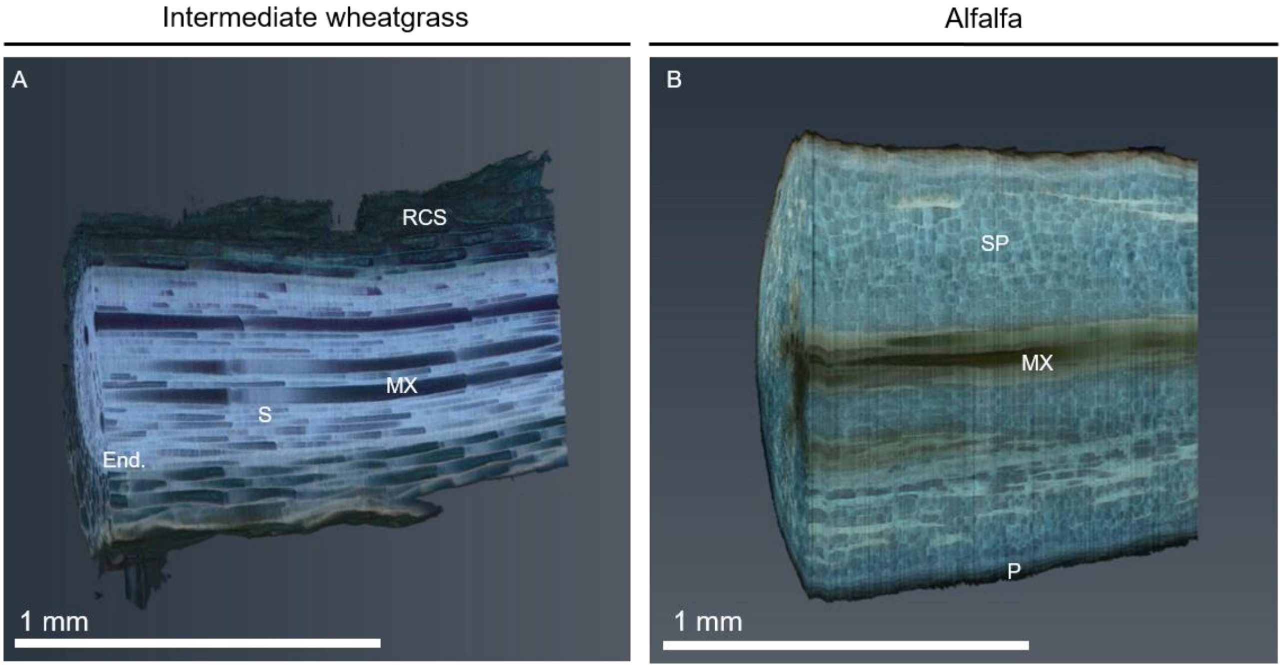
Supplemental Table 2. Composition of the solution culture (Half-Hoagland (Hoagland and Arnon 1950)*)*

Nutrient	Molar concentration (mol/L)
Potassium nitrate (KNO3)	1
Calcium nitrate (Ca(NO3))	1
Ammonium phosphate ((NH4)2PO4)	1
Magnesium sulfate (MgSO4)	1
Boric Acid (H3BO3)	25
Manganese chloride (MnCl2)	2
Zinc sulfate (ZnSO4)	2
Cupric sulfate (CuSO4)	0.5
Molybdic acid (H2MoO4)	0.5
Ferric EDTA (Fe-EDTA)	20

Supplementary figures



Supplemental Figure 1. Evolution of root anatomical phenes along lateral roots of a 1-m long nodal root of intermediate wheatgrass (A-E; left panel) and lateral roots of a 1-m long axial root of alfalfa (F-J; rigth panel) grown in mesocosm on a sandy loam and a loamy soil, respectively. (A;F) Cross-sectional area, (B;G) root cortex ratio, (C;H) number of metaxylem vessel (D;I) metaxylem vessel diameter and (E;J) total conductance. Average value ± standard error. Comparisons were done at each soil depth between the two soil types. Asterisks indicate significance differences, respectively with * $p \leq 0.05$, ** $p<0.01$ and *** $p<0.001$. P values indicate results from F-test



Supplemental Figure 2. Three-dimensional reconstruction of intermediate wheatgrass nodal (A) and alfalfa 1st order lateral root (B). Endodermis (End), MetaXylem (MX), Cortex area (C), Stele area (S), Periderm (P), Secondary Parenchyma (SP) and Root Cortical Senescence (RCS)

Comparing the deep root growth and water uptake of intermediate wheatgrass and alfalfa.

Status

Ready for submission to Plant and Soil (approved by all co-authors and pre-reviewed by Kirsten Verburg).

Authors list

Corentin Clément[1], Joost Sleiderink[1&2], Simon Fiil Svane[1], Abraham George Smith[1], Efstathios Diamantopoulos[1] Dorte Bodin Desbrøll[1], Kristian Thorup-Kristensen[1]

[1]*Department of Plant and Environmental Science, University of Copenhagen, 1871 Frederiksberg, Denmark*

[2] *Centre for Crop Systems Analysis, Wageningen University and Research, 6708PB Wageningen, The Netherlands*

Abstract

Water is the most important yield-limiting factor worldwide and drought is predicted to increase in the future. Perennial crops with more extensive and deep root systems are interesting for accessing deep stored water, building resilience to water shortage and intensifying sustainable cropping systems. In the context of human nutrition, perennial grain crops are very interesting. However, it is questionable whether they are as effective in using subsoil water as other perennial crops. We compared intermediate wheatgrass *Thinopyrum intermedium*, a perennial grain crop, and alfalfa *Medicago sativa*, a perennial forage crop, for subsoil root growth and water uptake.

Using TDR sensors, stable isotope labelled water, minirhizotrons and the Hydrus-1D model we characterised the root distribution and water uptake patterns of these two perennial crops during two cropping seasons under field conditions down to 2.5 m soil depth.

Both crops grew roots down to 2.0 m depth that were active in water uptake but alfalfa was deeper rooted than intermediate wheatgrass. All experimental methods concluded that alfalfa used more water from below 1.0 m depth than intermediate wheatgrass. However, simulations predicted that intermediate wheatgrass used more than 20 mm of water after anthesis from below 1 m soil depth. Both crops were particularly water stressed in 2018 in comparison to 2019. Simulations confirmed the advantage of having deep roots to access deep stored water in case of drought.

In regions with high groundwater recharge, deep-rooted perennial crops, in particular perennial grain crops, have great potential to increase the resilience of cropping systems to water scarcity.

Introduction

Water is the most yield-limiting factor globally and water scarcity will increasingly constrain food production in the future due to climate change and increased human water consumption (Rosegrant et al. 2009; Kummu et al. 2016). In Europe, increased evapotranspiration and a decrease in rainfall events in summer are expected to cause major water limitations, affecting both rainfed- and irrigated-agricultural systems (Turral et al. 2011; Dezsi et al. 2018; Rasmussen et al. 2018; Toreti et al. 2019). Moreover, sustainable intensification of agricultural systems is becoming a priority to meet increasing food demand and limit their associated environmental costs (Hunter et al. 2017; Fischer and Connor 2018). Part of this challenge is to find adaptation strategies that will increase the efficiency and resilience of agricultural systems regarding water usage.

In this quest, exploitation of deep soil resources has been neglected despite the advantages that increased rooting depth confers to plants and agricultural systems (Lynch and Wojciechowski 2015; Thorup-Kristensen et al. 2020a). Due to their extensive root systems, year-round ground cover and lower input requirements, perennial crops are drawing more attention (Cox et al. 2006; Crews and Dehaan 2015). Due to these characteristics, perennial cropping systems tend to provide ecosystem benefits such as soil carbon sequestration, reduced nutrient leaching and increased subsoil water uptake (McIsaac et al. 2010; Culman et al. 2013) compared to annual cropping systems (Pimentel et al. 2012; Crews et al. 2016). However, most of the perceived benefits of perennial farming systems derive from studies on forage and native grassland as discussed by Culman et al. (2013). With 80% of the human diet being cereals and oil seed crops, the development of perennial grain crops is attracting particular interest (Cox et al. 2006; FAO 2013; Crews and Dehaan 2015). Therefore, particular efforts are needed to (1) understand the conditions required for perennial crops to achieve the above mentioned benefits, (2) unravel the potential of perennial grain crops and (3) assess the compatibility of perennial farming with farmers practices (FAO 2013; Marquardt et al. 2016; Dehaan et al. 2018).

Deep subsoil water acquisition has led to substantial yield increase under drought in crops such as wheat (Manschadi et al. 2006; Kirkegaard et al. 2007; Cutforth et al. 2009), rice (Fukai and Cooper 1995) maize (Kondo et al. 2000) sorghum and sunflower (Bremner and Fazekas de St. Groth 1986). Deep rooting is considered to be a key trait for drought avoidance (Hund et al. 2009; Lynch 2013). However, the sole presence of deep roots is not automatically an advantageous trait. First, the presence of roots in moist subsoil have been shown to be a poor indicator of their activity, especially under water stress (Prechsl et al. 2015; Rasmussen et al. 2019). Second, as deep rooting is generally associated with more extensive water exploitation, in water-limited environments, a more conservative water use could be advantageous to saving water during earlier stages for later use (Richards and Passioura 1989; Schoppach et al. 2013). Nonetheless, in environments where water has been historically in excess and available in the subsoil, such as northern Europe, growing deeper-rooted crops can increase resilience to future climate fluctuations to sustain agricultural production. Benefits from deeper rooted crops must be considered in the context of the farming systems and management practices (Lilley and Kirkegaard 2016; Thorup-Kristensen and Kirkegaard 2016).

Along the soil-plant-atmosphere continuum, water moves passively from areas of high water potential (i.e. the soil) to areas of low water potential (i.e. the atmosphere) along a water potential gradient set up by transpiration (for a review see McElrone et al. (2013)). Due to the complexity of the hydraulic resistances to water flow that occur along the soil-plant system and their spatial and temporal variability it is difficult to make assumptions on root water uptake, see reviews by Lobet et al. (2014) and Ahmed et al. (2018). First, the movement of water in the soil, within the rhizosphere and the plants is governed by non-linear functions that vary according to the plant and environmental conditions. Second, plants develop physiological responses to soil drying and increase in vapor pressure deficit, which are highly variable in time and control the plant water uptake such as transpiration regulation (Vadez et al. 2014), mucilage exudation (Ahmed et al. 2014), compensated root growth (Vandoorne et al. 2012) or soil structure exploitation (e.g. clumping of roots in macropores (White and Kirkegaard 2010).

Understanding root growth and functioning under field conditions is thus particularly challenging due to the complex soil-plant-atmosphere interactions and even more challenging with increasing soil depth (Maeght et al. 2013). Research on deep roots is not common, especially due to the time, labor and costs involved (Thorup-Kristensen et al. 2020a) but recent advances in sensor technology, stable isotope techniques and imaging devices offer exciting perspectives. In addition coupling empirical measurements with predictive models offers interesting avenue for understanding the processes involved at great soil depth (Lilley and Kirkegaard 2011; Mazzacavallo and Kulmatiski 2015; Zheng et al. 2018) and will likely overcome methodological restrictions and biases such as destructive sampling, difficulty in obtaining in-situ and repeated chronological measurements.

Alfalfa, *Medicago sativa,* is a common perennial legume cultivated as a forage crop and used in crop rotations to enhance soil structure and rebuild nitrogen content (Angers and Caron 1998). Due to its extensive root growth, alfalfa is known for its deep water use (Angus et al. 2001; McCallum et al. 2001; Ward et al. 2002). Among perennial grain crops that offer worthy prospects for adoption, intermediate wheatgrass, *Thinopyrum intermedium,* has been attracting international attention (FAO 2013). It has long been used as a forage crop but also produces edible seeds (Dehaan et al. 2018). As a relative to wheat, it offers good hybridization perspectives as well as market and nutritional opportunities (Becker et al. 1991; Dehaan et al. 2018). While there are indications that intermediate wheatgrass is deep rooted (Cox et al. 2006), little is known about the extent of its rooting system and root activity below 1.5 m depth.

Therefore, this study focuses on two research questions: (1) how does root distribution of intermediate wheatgrass vary with soil depth? and (2) how effective are roots of intermediate wheatgrass in using deep soil water? Performances of intermediate wheatgrass where compared to alfalfa due to its deep root system and well-known capacity of using deep soil water. Field investigation combined soil moisture content sensors, water stable isotope methods and minirhizotron imaging to characterize root distribution and water uptake patterns down to 2.5 m soil depth. Quantitative estimation of the contribution of deep soil layers to crop transpiration was done using the Hydrus-1D model. Comparison of the advantages, disadvantages and degree of uncertainty of the different research methods in predicting root growth and water uptake constituted an underlying objective of this study.

Material and Method

Site description and meteorological data

The experiment was conducted at the experimental farm of the University of Copenhagen (55.66815°N, 12.30848°E). Climate at the study site was a marine west coast climate (Cfb according to the Köppen climate classification) and local weather conditions were obtained by an on-site weather station (Svane et al. 2020). With 100 mm less rainfall and 67 mm higher reference evapotranspiration during the growing season, 2018 was a drier year than 2019 (Fig. 1). In May, June and July 2018 rainfall was consistently lower than the 56-year 3rd quartile, making 2018 a very dry year.

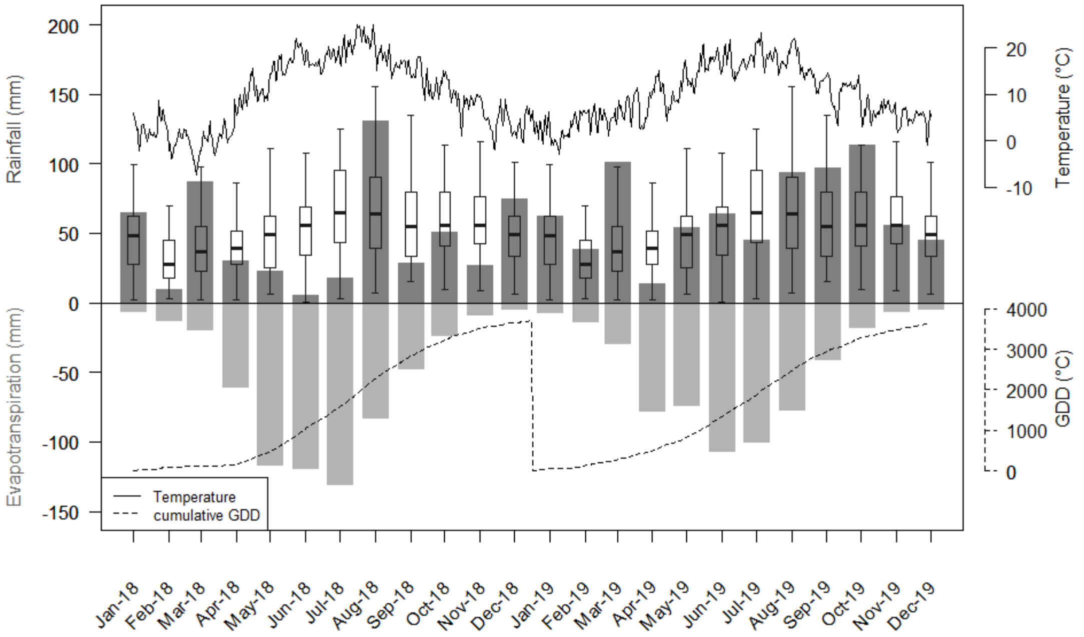


Figure 1. Monthly rainfall (dark grey bars) and reference evapotranspiration (light grey bars). Daily average temperature (solid-line) and cumulative Growing Degree Day (GDD) calculated by the method of (Baskerville and Emin 1969) with base of 0 ℃ (dashed-line) at Taastrup, Denmark, from January 2018 to December 2019. Boxplot: Monthly rainfall variability from 1962 to 2008. (Source: (Svane et al. 2020))

The soil, a haplic Luvisol according to the FAO soil classification system (FAO 2006), changes from a sandy loam with approximately 15% of clay in the topsoil to a loam below 0.25 m with approximately 20% of clay (Table 1). The subsoil was characterised by the presence of calcareous till, generally found below 1 m soil depth. Soil bulk density was 1.4 g/cm^3 in the topsoil and increased to 1.8 g/cm^3 in the subsoil. At 0.75, 1.5 and 2.5 m soil depth, water retention was measured using the standard sand box and pressure plate method (Table 1; Supplemental Fig. 1).

Table 1. Soil physico-chemical properties at the studied site. Average values with standard error in brackets. Note: texture classification follows the Danish texture standard. (TOC) Total Organic Carbon, (N) Nitrogen content, (P) Phosphorus content, (K) Potassium content, (Mg) Magnesium content, (FC) Field Capacity, (PWP) Permanent Wilting Point. (^{1}) n=5, (^{2}) n=10

Soil depth	Clay^1 <2µm	Silt^1 2µm-200µm	Sand^1 200µm-2.0mm	TOC^1	pH^1	N^1	P^1	K^1	Mg^1	FC^2	PWP^2	Soil bulk Density^2	Porosity^2
m	%	%	%	%		%	mg/100g	mg/100g	mg/100g	Vol %	Vol %	g/cm^3	%
0-0.25	14.6 (0.2)	16.1 (1.4)	67.9 (1.5)	0.9 (0.3)	7.7 (0.1)	0.10 (0.02)	4.7 (0.5)	11.5 (0.6)	7.3 (0.8)	NA	NA	1.43 (0.03)	45.9 (0.01)
0.75	20.0 (1.1)	14.6 (0.8)	64.8 (1.7)	0.4 (0.1)	7.8 (0.1)	0.05 (0.01)	1.1 (0.2)	8.3 (0.5)	8.4 (0.9)	27.0 (0.5)	12.3 (1.3)	1.74 (0.003)	34.4 (0.001)
1.5	19.5 (0.9)	18.2 (0.8)	62.1 (1.6)	0.1 (0.01)	8.0 (0.1)	<0.03 (.)	0.6 (0.1)	6.6 (0.3)	7.4 (0.3)	30.5 (0.7)	10.1 (0.4)	1.75 (0.003)	33.8 (0.001)
2.50	20.0 (0.6)	19.3 (0.7)	60.7 (0.7)	0.1 (0.01)	8.0 (0.1)	<0.03 (.)	0.4 (0.0)	6.9 (0.6)	8.6 (1.2)	27.2 (0.2)	12.8 (0.4)	1.83 (0.003)	30.9 (0.001)

Plant material, establishment and management

Intermediate wheatgrass *Thinopyrum intermedium* (5th selection cycle, Land Institute, Kansas, USA) and alfalfa *Medicago sativa* (cv. Creno) were sown on 11 April and 9 September 2016, respectively on three experimental plots of 100 m^2, kept under rainfed conditions. Intermediate wheatgrass was sown at 20 kg seeds/ha with a row spacing of 25 cm. Alfalfa was sown at 20 kg seeds/ha in strips (1.5 x 10m), 3 m apart, as part of an intercropping experiment with spring barley (2017) and intermediate wheatgrass (2018 - 2019). Plants received 100 kg N/ha in the form of NPK fertilizer (YaraMila 21-7-3) in early spring, every year. Crops were grown organically, mechanical weed management was carried out when necessary and no insect and disease management was performed. Alfalfa was harvested three times per year using a grass harvester, whereas intermediate wheatgrass was harvested for grains at maturity, using a combine harvester.

Measurements and monitoring

- *Crop growth, yield and stomatal conductance*

During the main growing season (Apr.-Oct.), crop height and phenological stages were recorded every two weeks. Stomatal conductance was also measured every two weeks on the upper and lower part of intact green leaves on a sunny day, using a portable porometer SC-1 (Decagon, USA). Measurements were done at 8h-10h ("Morning"), 11h-13h ("Midday"), and 15h-17h ("Afternoon") and daily average values were calculated. Sub-samples of grains and biomass were oven-dried at 80°C during 48 hours for estimation of moisture content and Thousand Kernel Weight (TKW) was calculated.

- *Soil water content*

Soil Volumetric Water Content (VWC) was monitored, as a proxy of root water uptake, using TDR sensors (Acclima 310S with a 0.1% resolution, Acclima, Inc., USA). In each research plot, three replicated sensors were installed at 0.75, 1.5 and 2.5 m depth. TDR sensors were mounted on PVC pipes and inserted vertically down a pre-drilled hole. The holes were refilled with bentonite to avoid any preferential flow along the edge of the pipe. All sensors were at least 3 m from the plot edge and 1 m apart. The sensor's cables were protected with PVC pipes and buried 0.3 m deep to protect them from rodents and to allow the use of agricultural machinery. Groundwater level was monitored using a CTD-10 sensor (Decagon, USA) in two boreholes located on the research plots. Two CR6 data loggers (Campbell Scientific, Inc., USA) collected data from the sensors at one-hour intervals.

- *Root growth*

Seasonal root growth was monitored using 5-m deep minirhizotrons placed at a 30° angle from the vertical. Minirhizotron tubes were imaged on a monthly basis from May to August in 2018 and 2019 using a single multispectral camera unit mounted on an automatic minirhizotron image acquisition system (Svane et al. 2019a). Root detection was performed using an automatic image segmentation method utilising a convolutional neural network (Smith et al. 2020). To validate the procedure, 2000 images were randomly selected from the entire database of images taken. Decision was taken to train the network on all root structures without distinction of living or senescing roots. Predictions of the average root length per image by the neural network were compared to average root intensity per image obtained by the grid counting method as in Thorup-Kristensen et al. (2012). Both crops had an R-square higher than 0.55, which was considered sufficient for the purpose of this study (Supplemental Fig. 2). Extra care was taken to make sure that the network would not create any false positives in the lower range of root intensity as these could significantly affect the crop rooting profile at depth. Maximum rooting depth was defined as the averaged depth of the three deepest images with a root length greater than 0.6 cm.

- *Stable isotope experiment*

Water stable isotope methods (i.e. natural abundance and deuterium labelling) were used to derive direct measurements of root water uptake at depth. A one-time measurement of root length density was obtained when soil cores were taken.

Background information and preparation

The water stable isotope experiment was divided in two periods, referred to as the June and the July-August period. On 13 June and 18 July 2019 respectively, one soil core was taken in each research plot, using a standard steel auger (ø7 cm, height 10 mm, 385 cm^3 internal volume, Eijkelkamp). Soil samples were taken from the soil surface to a maximum depth of 2.0 m in regular increments of 0.25 m and stored in zip-lock bags. For each soil depth increment, three sub-samples were taken to estimate soil volumetric water content (~300g), soil deuterium natural abundance (~20g) and root length density (~1.5 kg). Soil fresh weight was measured, and samples were kept frozen prior to analysis. In each drilled hole, a PVC pipe (2.1 m in length, ø6cm) was inserted. PVC pipes were open at the bottom to allow placement of a deuterium enriched solution at 1.9 – 2.0 m without contamination of upper soil layers. PVC pipes were perforated in their lower 10 cm, to ensure a good flow of the tracer solution into the soil. The upper part of each pipe was covered with a PVC plug and the space around the pipes was backfilled with bentonite to prevent water infiltration along the tube.

Root length density and soil volumetric water content.

In the laboratory, root samples were obtained by manually washing them free of soil using a sieve with a mesh size of 0.5 mm. For each soil sample collected, every root segment was retrieved from the sieve and stored at 4 °C in 70% ethanol. Roots were scanned using an Epson Perfection V700 Photo flatbed scanner at 800 dpi and analyzed for root length and mean diameter with WINRhizo software (Regent Instruments, Quebec, Canada). Soil samples were oven dried at 80°C for 48 hours for estimation of the gravimetric water content.

Stable isotope labelling and sampling

On 17 June and 22 July 2019, a deuterium enriched tracer solution was poured down the PVC pipes (δ^2H_{June}=300000‰, $\delta^2H_{July\text{-}August}$=600000‰). Separate PVC pipes were used for the two periods. Prior to each tracer injection, groundwater was sampled in the two boreholes. Rainfall water was collected in a bucket and sampled after every rain event. Soil sampling occurred four days prior to the tracer solution injection and plant sampling started on the day of injection and then occurred at 1, 4, 8, 16, and 24 days after injection. Plant biomass samples were collected around 10 am, stored in a capped test tube (Pyrex, 1622/22M; Kartell Labware, ø22mm) and frozen shortly after collection. In the laboratory, leaves and stems were separated in a cold chamber prior to water extraction to account for differences in isotopic signature. Plant transpiration water was collected in the morning by placing a transparent plastic bag ($20L_{alfalfa}$-$80L_{intermediate\ wheatgrass}$, Logicon Nordic A/S) over the plant shoots, tying the bag with a string creating a small gulley to prevent leakage of the transpiration water. Water was then sampled in the gulley in the afternoon around 2-3 pm. Plant biomass and transpiration water samples were taken near the tracer injection pipes and in non-impacted areas (i.e. control - natural abundance). Date and types of samples processed are presented in (Supplemental Table 1).

Stable isotope analysis

Water in leaf, stem and soil were extracted using a cryogenic vacuum distillation as described by Ehleringer and Osmond (1989). Transpiration water samples were filtered using filter syringe (0.45µm, FisherSci). All water samples were put into glass vials prior to analysis. Stable isotopes (i.e. δ^2H and $\delta^{18}O$) in water samples were analyzed using an isotopic water analyzer (L2140-i, Picarro Inc., USA). All water extraction and analyses were performed at the SilvaTech Platform, INRAE-Nancy, France. Isotope values are expressed in delta notation and calculated as followed (equation 1):

$$(1) \qquad \delta\ (‰) = \frac{Rsample}{Rstandard} - 1\ \times 1000$$

Where Rsample and Rstandard are the ratios of the heavier to the lighter isotope of the sample and of the standard (i.e. Vienna Standard Mean Ocean Water, VSOW), respectively. The regression between δ^2H and $\delta^{18}O$ values of monthly rainfall sampled in Taastrup, between November 1965 and June 1971 describes the local meteoric water line (LMWL) and were obtained from a public database (https://nucleus.iaea.org/wiser). The LMWL is used as a reference line that determines the natural isotope ratio of ^2H and ^{18}O at the study site. Any deviation from this reference line indicates a change in the isotope ratio of the water and that heavy isotope discrimination has taken place (Clark and Fritz 1997).

Modelling approach

Simulation study was used to assess the relative root water uptake from different soil depths as well as specify seasonal differences.

- *Model description*

The HYDRUS-1D model (version 4.17; www.pc-progress.com) was selected to simulate the 1-dimensional flow of soil water under saturated and unsaturated conditions and plant water uptake (Šimůnek et al. 2009). HYDRUS-1D numerically solves the Richards equation under variable soil water conditions with a sink term that represents plant water uptake. Main equations behind the model functioning are provided in supplementary material "Hydrus-1D Model functioning and equations". For more information on Hydrus-1D see (Šimůnek et al. 2009).

- *Parametrisation of Hydrus-1D*

The model was run for the years 2018 and 2019 with atmospheric conditions as upper boundary conditions. Reference evapotranspiration was calculated on an hourly basis using the Penman-Montheith equation (Allen et al. 2006). Crop potential evapotranspiration was calculated by applying a crop factor to the reference evapotranspiration (Allen and Pereira 1998). Hydrus-1D requires potential soil evaporation and crop transpiration to be specified as separate inputs. To do so, the Beer's law method was used to partition potential evapotranspiration into potential transpiration and evaporation fluxes based on the leaf area index (Sutanto et al. 2012). Leaf area index was calculated based on crop height for alfalfa and crop stages for intermediate wheatgrass using equations from Allen et al. (1989) and Mitchell et al. (1998), respectively (Supplemental Table 2). A variable pressure head was used as lower boundary conditions based on the measured groundwater level. Calculation methods of actual transpiration, evaporation and root water uptake are provided in supplementary material "Hydrus-1D Model functioning and equations". Initial conditions were set to complete saturation of the soil profile, at the beginning of each year, which is normally the case at the study site (Fig. 4).

The soil profile was set to 2.5 m and divided in two simplified layers (i.e. top layer: 0-0.75 m and bottom layer: 0.75-2.5 m). According to equation 4 and 8 (supplementary material "Hydrus-1D Model functioning and equations"), the total number of parameters describing soil hydraulic properties are equal to 18, 9 for each layer. Parameters for the matrix region $(\theta_s, \theta_r, \alpha_m, n_m)$, 8 in total, were fixed by fitting the measured water retention curves (Supplemental Fig. 1). The remaining 10 parameters $(2x[K_s, \tau, \alpha_f, n_f, w_f])$ were estimated by means of inverse modelling (Hopmans and Šimůnek 1999) and by minimising the objective function: (2)

$$O(p) = \sum_{i=1}^{nn} \left(\theta_{exp} - \hat{\theta}\right)^2$$

where p is the unknown parameter vector, $[K_s^{top}, \tau^{top}, a_f^{top}, n_f^{top}, w_f^{top}, K_s^{bot}, \tau^{bot}, a_f^{bot}, n_f^{bot}, w_f^{bot}]$, nn is the total number of TDR measurements at three depths, and θ_{exp} and $\hat{\theta}$ are measured and simulated water content values, respectively. Superscripts top and bot refer to top and bottom soil layers. Parameter adjustment was performed for each crop type and compensation level to improve the fit of each model assuming subtle soil differences related to soil texture and cropping history (Rasse et al. 2000). We used the Levenberg-Marquart algorithm (Marquardt 1963) for minimising equation 2, as implemented in Hydrus-1D. Parameter uncertainty was estimated by using the first-order second moment method (Diamantopoulos et al. 2012).

Root water uptake parameters were obtained from an existing database, Taylor and Asshcroft (1972) for alfalfa and parameters for wheat were obtained from Wesseling (1991) and applied to intermediate wheatgrass. Respective values are summarized in Table 2. Crop rooting profile was fixed for the whole simulation based on the average root length density obtained from the soil coring method in 2019.

In Hydrus-1D a compensatory water uptake function is used to reflect compensatory water uptake that occurs under topsoil drying (Jarvis 1989; supplementary material "Hydrus-1D Model functioning and equations"). Little is known about the compensatory water uptake threshold ($\omega_{c)}$ and how it varies between crops (Skaggs et al. 2006). Decision was taken to run the model for different values of ω_c found in the literature (i.e. 0.1, 0.75, 0.875 and 1.0) and discuss the relevance of the results and of the compensation effect (Skaggs et al. 2006; Simunek and Hopmans 2009; Mazzacavallo and Kulmatiski 2015). For each crop type (i.e. alfalfa and intermediate wheatgrass), four compensation scenarios (i.e. 0.1, 0.75, 0.875 and 1.0) were investigated. A total of 8 simulations were carried out. All fixed and fitted parameters are listed in Table 2 and supplementary Table 3.

Table 2. Crop factors, compensation factor (ωc), feddes parameters, and soil hydraulic parameters for the two layers. Parameters starting with * have been estimated by inverse modelling and standard errors are provided. Number 1 and 2 associated to the soil hydraulic parameters refers to the top and bottom soil layer, respectively

Category	Parameter	Unit	Alfalfa	Intermediate Wheatgrass
Crop factor	Ini	-	0.4	0.7
	Mid	-	1.2	1.15
	End	-	1.15	0.25
	ω_c	-	0.75	0.75
Feddes parameter	H1	cm	-10	0
	H2	cm	-25	-1
	$H3_{high}$	cm	-1500	-500
	$H3_{low}$	cm	-1500	-900
	H4	cm	-8000	-16000
Soil hydraulic parameters	$1\text{-}\theta_s$	-	0.312	0.307
	$1\text{-}\theta_r$	-	0	0
	$1\text{-}\alpha_m$	cm^{-1}	0.0055	0.0055
	$1\text{-}n_m$	-	1.19	1.19
	*1-Ks	cm.day^{-1}	12.27±1.7	0.37±1.1e^{-2}
	*1-L	-	0.12±0.1	0.01±7.7e^{-4}
	$^*1\text{-}W_f$	-	0.12±6.1e^{-3}	0.11±2.3e^{-3}
	$^*1\text{-}\alpha_f$	cm^{-1}	0.12±1.2e^{-2}	0.10±3.8e^{-4}
	$^*1\text{-}n_f$	-	3.90±0.8	4.92±0.1
	$2\text{-}\theta_s$	-	0.289	0.320
	$2\text{-}\theta_r$	-	0	0
	$2\text{-}\alpha_m$	cm-1	0.0013	0.0013
	$2\text{-}n_m$	-	1.37	1.37
	*2-Ks	cm.day^{-1}	0.90±6.1e^{-2}	1.67±0.2
	*2-L	-	10.00±1.2	3.49±0.6
	$^*2\text{-}W_f$	-	0.02±1.5e^{-3}	0.05±8.5e^{-3}
	$^*2\text{-}\alpha_f$	cm^{-1}	0.10±4.5e^{-3}	0.10±1.2e^{-2}
	$^*2\text{-}n_f$	-	6.76±1.1	1.71±0.2

Data analysis

All statistical tests were performed using the *R* software using the Rstudio interface (version 1.1.383; *R* core team, 2018) using the "lme4, version 1.1-21", "multcomp, version 1.4-10", "ggplot2, version 3.1.0" and "emmeans, version 1.4.3.01" packages. We used linear mixed models to look for variations in soil volumetric water content, root length, root length density and soil deuterium natural abundance with soil depth. Normality and homoscedasticity of the data were checked by looking at the residuals and Q-Qplot. When data did not fulfill these two assumptions, a log transformation was applied to normalize the data. Differences between stomatal conductance, biomass, grain yield, TKW, water samples δ^2H and $\delta^{18}O$ between crops and sample types were tested using pairwise comparisons of means with Tukey-adjusted p-values.

Results

Crop performance

Although exposed to different climatic conditions, we did not find any significant differences in grain yield between the two years for intermediate wheatgrass although differences in TKW were observed (Table 3). For alfalfa, harvested biomass was significantly lower in June 2018 than 2019 but no differences were found for the August and October harvest (Table 3). Stomatal conductance was higher in alfalfa than in intermediate wheatgrass in both years (Fig. 2). Alfalfa had lower stomatal conductance in June, July and August 2018 compared to 2019 whereas intermediate wheatgrass presented lower stomatal conductance in June 2018 compared to 2019. Overall, these results suggest that both crops were more water stressed during the spring-summer 2018 compared to 2019.

Table 3. Comparison of straw biomass, grain yield and thousand kernel dry weight (TKW) of intermediate wheatgrass and harvested biomass of alfalfa in two seasons. Average values with standard error in brackets. Comparisons were made between years, letters that differ indicate significant differences at (p<0.05). *Intermediate wheatgrass straw biomass does not reflect the actual straw yield as measurement errors were introduced by the combine harvester. However, yearly comparisons remain possible assuming that errors were constant across years

	Year	Harvest	Biomass (ton/ha)*	Grain yield (ton/ha)	TKW
Intermediate wheatgrass	2018	-	4.2 (0.4) (a)	0.84 (0.1) (a)	7.1 (0.05) (a)
	2019	-	3.8 (0.1) (a)	1.04 (0.1) (a)	5.9 (0.05) (b)
Alfalfa	2018	June	6.9 (0.6) (a)	-	-
	2019		8.1 (0.4) (b)	-	-
	2018	August	5.8 (0.6) (a)	-	-
	2019		5.5 (0.3) (a)	-	-
	2018	October	2.5 (0.1) (a)	-	-
	2019		2.2 (0.1) (a)	-	-

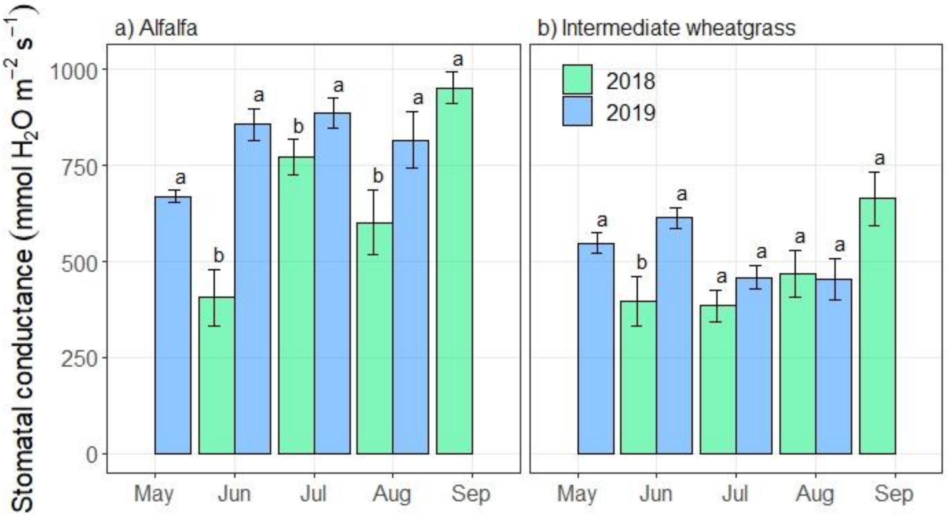


Figure 2. Daily stomatal conductance of alfalfa (a) and intermediate wheatgrass (b) during two seasons of cultivation. Average value ± standard errors (n_{2018}=6; n_{2019}=8). Comparisons were made between year, letters that differ indicate significant differences at (p<0.05). Note. Data for May 2018 are incomplete

Development and intensity of root growth

Both crops developed a deep root system (>2.0m) (Fig. 3). Maximum rooting depth from minirhizotron measurements was estimated at 2.0 m in August 2018 for both crops and at 2.8 and 2.5 m in August 2019 for alfalfa and intermediate wheatgrass, respectively (Fig. 3d). Along minirhizotron tubes, root length of intermediate wheatgrass decreased with soil depth whereas root length of alfalfa peaked between 0.75 and 1.75 m depth. In 2018, intermediate wheatgrass had significantly greater root length at 0.5, 1.0 and 1.25 m soil depth in May and at 1.0 m soil depth in June compared to 2019. No differences were found in July, but greater root length was observed at 0.25 m soil depth in August 2018 compared to 2019. Alfalfa had significantly lower root length in 2018 compared to 2019, particularly within the first 1.5 m depth of soil. Significant differences were observed during the months of May, July and August. Interestingly greater root length was observed at 1.75 m soil depth in July and August 2019 compared to 2018. In both years, alfalfa had greater root length than intermediate wheatgrass in the 0.75-1.75 m soil depth interval.

Root length density, obtained from soil cores taken in July 2019, decreased with soil depth for both crops (Fig. 5a). Intermediate wheatgrass had more roots in the topsoil than alfalfa with significant differences at 0.25 and 0.75 m soil depth (Fig. 5a). Intermediate wheatgrass had more coarse roots (i.e. diameter > 1 mm) in the topsoil layer (i.e. 0.25 m) while alfalfa had a higher number of coarse roots at depth (i.e. >0.5 m) with significant differences at 0.5 m and 1.0 m soil

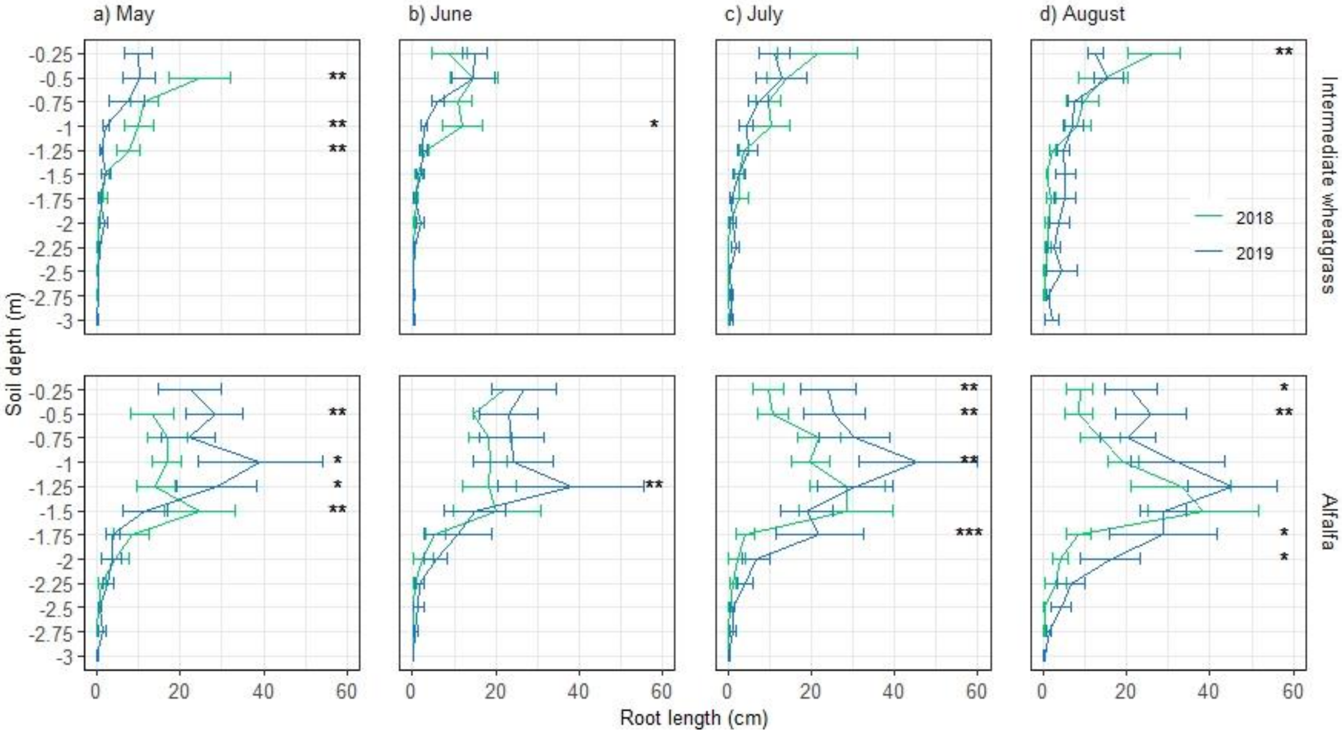


*Figure 3. Monthly root length distribution of intermediate wheatgrass (top row) and alfalfa (bottom row) as a function of soil depth in May (a), June (b), July (c) and August (d) 2018 (green) and 2019 (blue). Note: In May 2018, minirhizotron images at 0.25 m soil depth were not collected due to a technical issue with the camera. Asterisks indicate significance differences, respectively with * p<0.05, ** p<0.01 and *** p<0.001*

depth (Fig. 5b). Both crops had similar root length density in deep soil layers with on average 0.4 cm/cm^3 between 1.5 and 2.0 m, but alfalfa tended to have higher root length density at 1.75 and 2.0 m. However, no statistical differences were apparent and root length density was highly variable at those depths.

Soil moisture patterns

Both seasons gave similar results in terms of dynamics of soil moisture content at 0.75, 1.5 and 2.5 m but crop differences were identified (Fig. 4ab). In 2018, soil water content at 0.75 m soil depth started to decrease in June for both crops. Water depletion was relatively fast and by July this soil layer was dry (~18% VWC; pF>3.0) under both crops. In 2019, soil water depletion at 0.75 m started approximately one month earlier in May. At 1.5 m soil depth, soil water depletion started around July but showed contrasting patterns between the two crops. Alfalfa depleted this soil layer down to 25% VWC (~pF=2.8) whereas under intermediate wheatgrass the soil water content never dropped below 29% VWC (~pF=2.0). As observed at 0.75 m, water uptake at 1.5 m soil depth under alfalfa started about a month earlier in 2019 during the month of June. In August 2018, soil moisture content at 0.75 m depth increased to 24% for both crops due to rainfall events. Soil moisture content further decreased until October under alfalfa but no decrease was observed under intermediate wheatgrass. These results show that both crops induced changes in soil water content down to 1.5 m depth but that alfalfa extracted more water at depth than intermediate wheatgrass. Small reductions in soil water content (~1-2% VWC) were observed at 2.5 m around July in both years for alfalfa but no soil water extraction was observed under intermediate wheatgrass. These observations were supported by groundwater levels measured near the study plots in mid-august (Fig. 4c). In a well adjacent to the alfalfa plots, the groundwater level dropped down to 3 m and more than 3.5 m in 2018 and 2019, respectively. In contrast, in a well near the intermediate wheatgrass plots, the groundwater level only dropped to 2.5 and 2 m depth in 2018 and 2019, respectively. During the winter period, groundwater dynamics under both crops were similar.

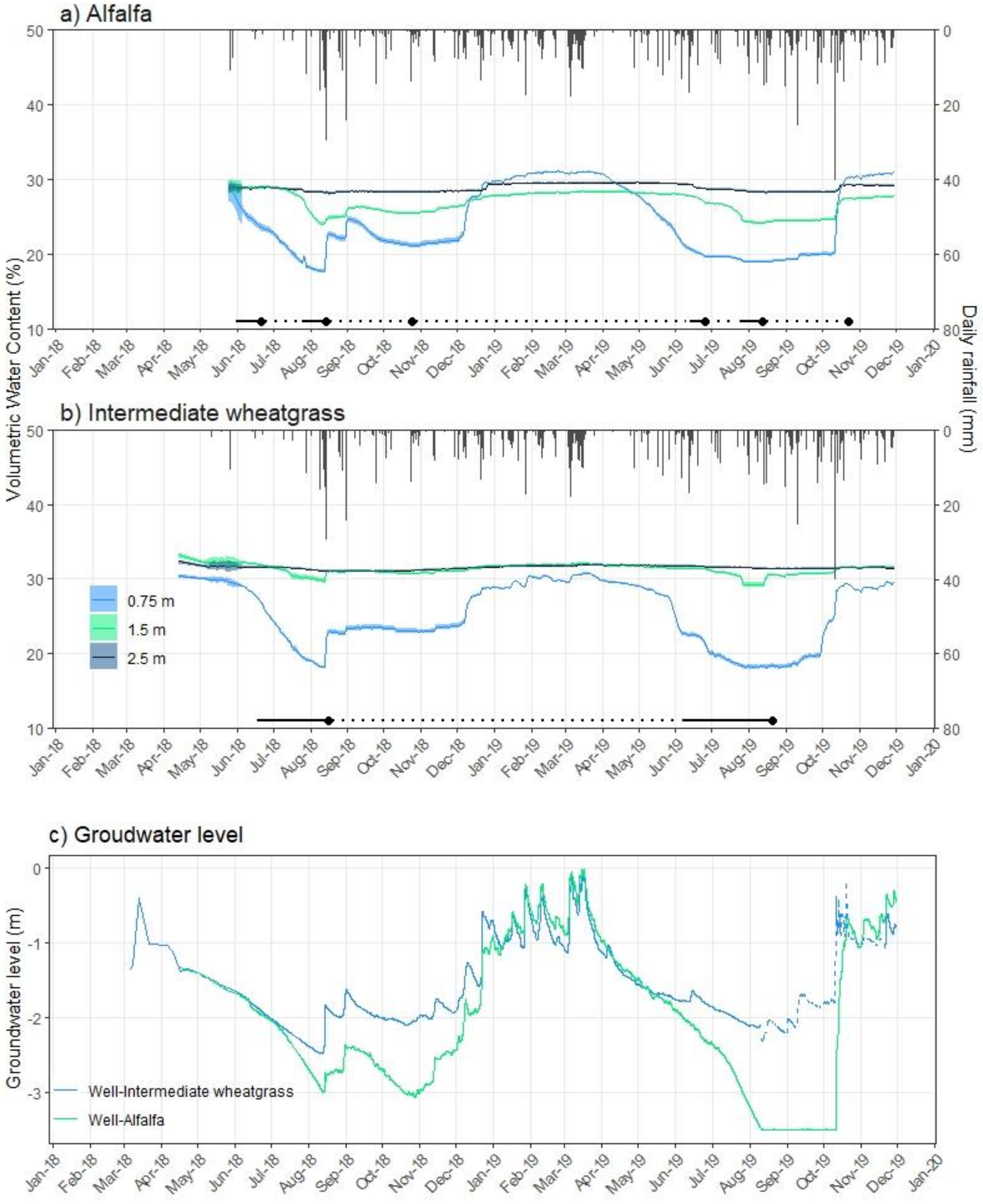


Figure 4. Soil volumetric water content evolution at 0.75 m (light blue), 1.5 m (green) and 2.5 m (dark blue) soil depth under alfalfa (a) and intermediate wheatgrass (b). Data covers two growing seasons from 01-05-2018 until the 30-11-2019. Daily average value (line) with corresponding standard error (shade) ($n_{alfalfa}$=6, $n_{intermediate\ wheatgrass}$ = 9). Grey bars indicates daily rainfall events. (─) indicates reproductive stage (i.e. flowering and grain filling), (…) indicates vegetative stage and (●) indicate harvest time. Groundwater level fluctuation in wells nearby alfalfa (green) and intermediate wheatgrass (light blue). Note: Dashed line indicates the use of data from a nearby well due to sensor failure (from the 11th of august until the 21st of November 2019)

Stable isotope experiment

- *Natural ^{2}H abundance of water*

The isotopic signature of the soil water varied with soil depth and aboveground crop type (Fig. 5c). Soil water isotopic signature was significantly more depleted under intermediate wheatgrass at 0.5 and 0.75 m soil depth compared to alfalfa. A similar though non-significant trend was observed at 1.5 m. Significant differences in soil water isotopic signatures seems to coincide with differences in root length density between the two crops (Fig. 5ac).

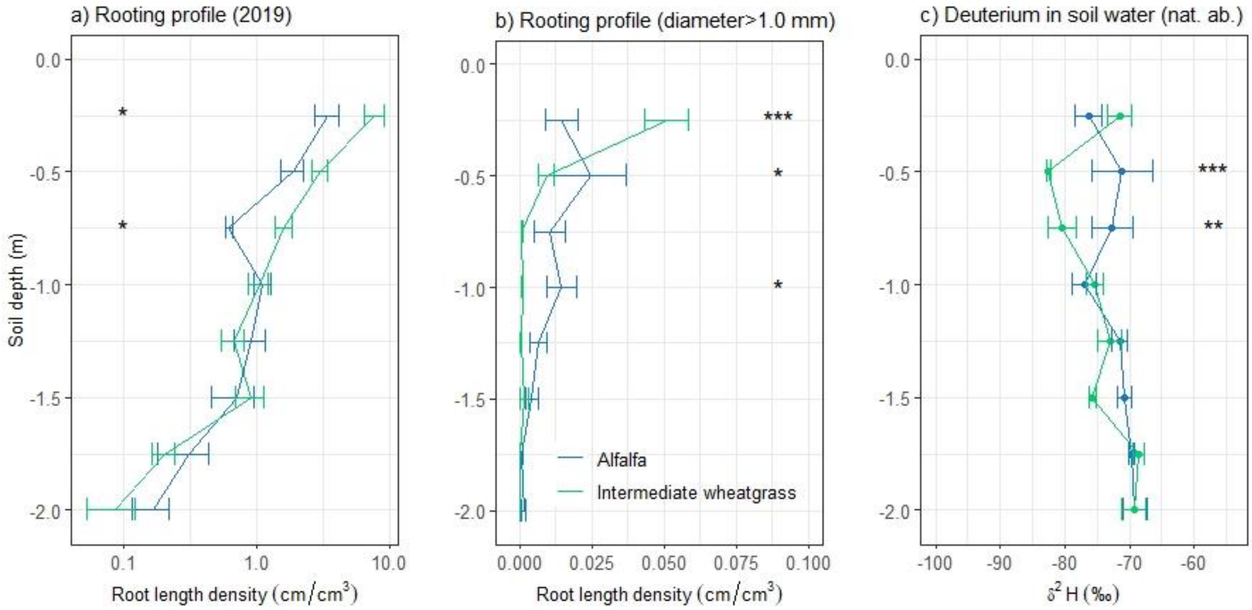


Figure 5. Root length density (a), root length density of root with a diameter > 1 mm (b) and natural abundance of δ^2H (c) evolution with soil depth of alfalfa (blue) and intermediate wheatgrass (green). Average value ± standard error. Data obtained by coring method and sampled on 27 of July 2019. Asterisks indicate significance differences, respectively with * $p<0.05$, ** $p<0.01$ and *** $p<0.001$

For four soil layers (i.e. 0.25, 0.5, 0.75 and 2.0 m), the correlation between root length density and soil water isotopic signature had R^2 values higher than 0.4 (Fig. 6a). The fact that correlation within soil layers is higher than the overall correlation (i.e. R^2=0.29 without soil layer distinction) for 6 layers out of 8, indicates a soil depth effect. The slope of the correlation is positive for the topsoil layer suggesting that, the higher the root length density the more enriched the topsoil is. However, below 0.25 m soil depth, the slope of the correlation is negative suggesting that a higher root length density would induce a depletion of the soil water isotopic signature. The slope of the regressions below 0.25 m soil depth gradually increases with increasing soil depth reinforcing the idea of a soil depth effect on the correlation. The correlation between root length density and soil moisture content was overall higher (R^2=0.69) with little effect of soil depth (Supplemental Fig. 3).

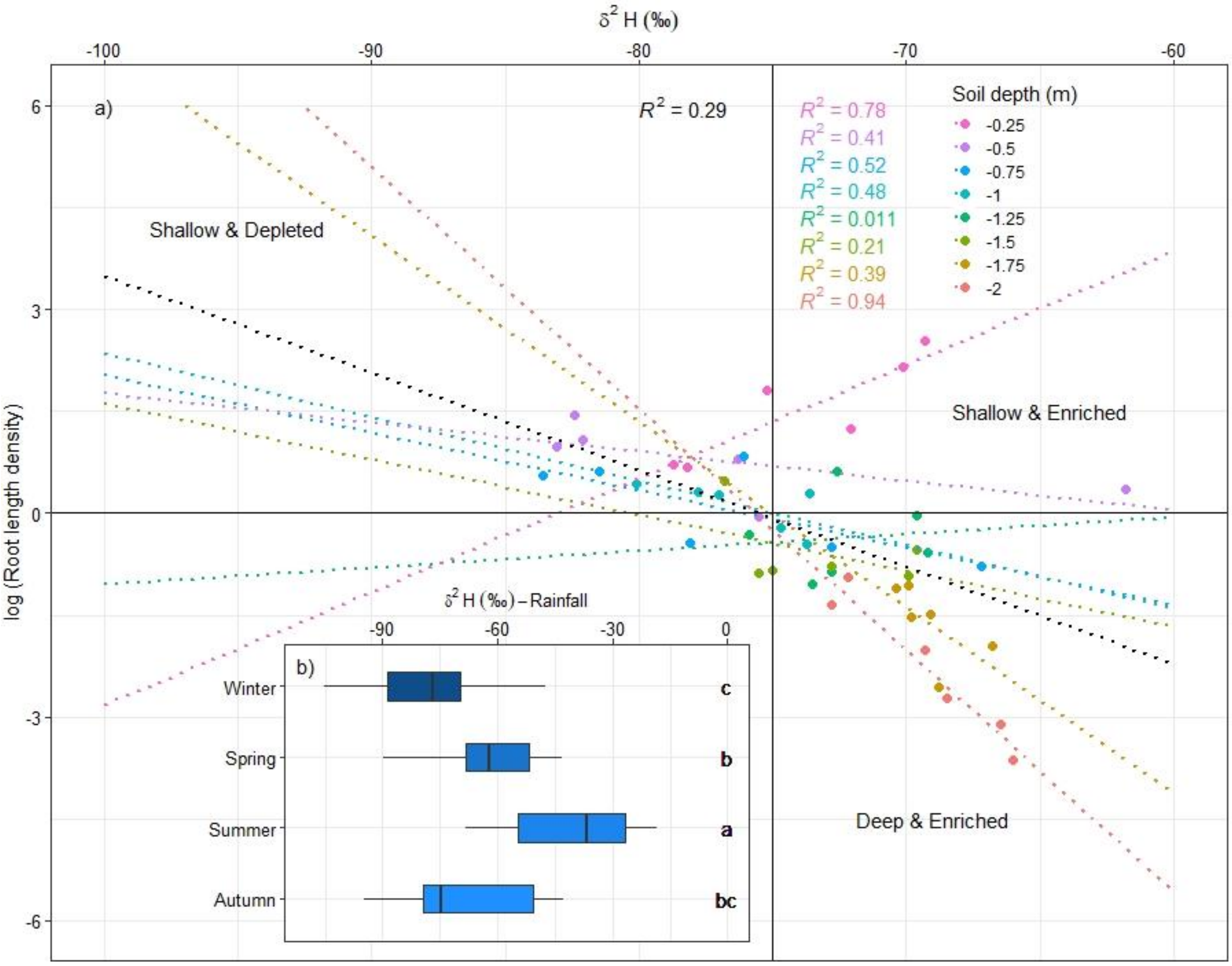


Figure 6. Correlation between average root length density (log) and average δ²H in soil water (a). Overall correlation is represented in black. Seasonal δ²H variations in rainfall (historical data) (b)

Across the different water samples collected, large variability occurred in their deuterium signatures (Fig. 7ac). When looking at the dual isotope plot (Fig. 7b) soil water and rainfall samples had slopes close to the LMWL, followed by transpiration water whereas groundwater, leaf and stem water samples differed greatly from the LMWL. Seasonal variations in the isotopic signature of rainfall were identified through analysis of historical rainfall data (Fig. 6b). Autumn and winter rainfall are usually more depleted (mean δ^2H = -67‰ and -79‰, respectively) while spring and summer rainfall are more enriched (mean δ^2H = -62‰ and -39‰, respectively).

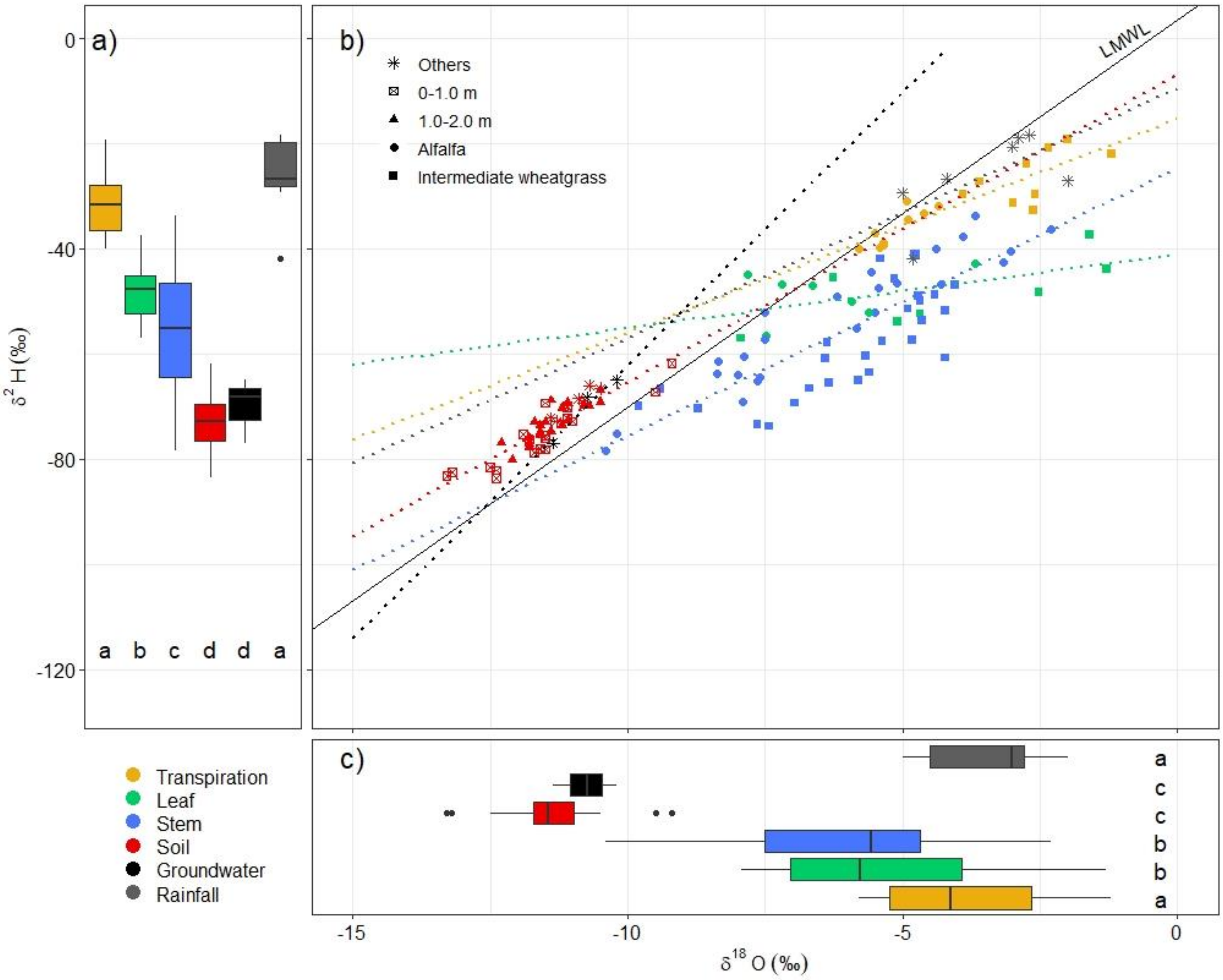


Figure 7. Dual isotope plot (i.e. regression between δ^2H and $\delta^{18}O$ values) for water samples originated from plant transpiration, leaves and stems as well as soil, groundwater and rainfall (b). Colors indicate origin of samples. For soil samples two depth categories are indicated by different symbols. The local meteoric water line is shown with a solid line (LMWL, $\delta^2H = 3.61 + 7.36 \times \delta^{18}O$). Sample type regression from measured isotopic ratios are shown with dashed lines. Boxplots for δ^2H (a) and $\delta^{18}O$ (c) grouped per sample type. Comparisons were made between sample types, letters that differ indicate significant differences at ($p<0.05$)

- *Labelling study*

In the labelling study, any samples with a positive isotope signature (i.e., more than twice the value of the third quartile of the control) were considered to reflect an uptake of deuterium tracer. Deuterium tracer uptake in alfalfa samples were observed on 21 and 24 June, on the 11, 27, 29 July and on the 7 August 2019 (Fig. 8a). Even though the deuterium tracer concentration was doubled during the July-August period, no increase in tracer uptake was detected on the samples' isotopic signatures. The highest deuterium signatures were obtained in leaf and stem samples (δ^2H=913‰ and δ^2H= 990‰, respectively) on the 24 June 2019. The deuterium isotopic signature of the samples was correlated to root length density below 1.5 m soil depth (Fig. 8b). In the case of intermediate wheatgrass, a positive isotopic signature (δ^2H=13‰) was observed solely on the 7th of August 2019. This highlights the capacity of intermediate wheatgrass to take up water from 2.0 m soil depth but in much smaller amounts than alfalfa (i.e. alfalfa's enrichment values were almost 100 times higher).

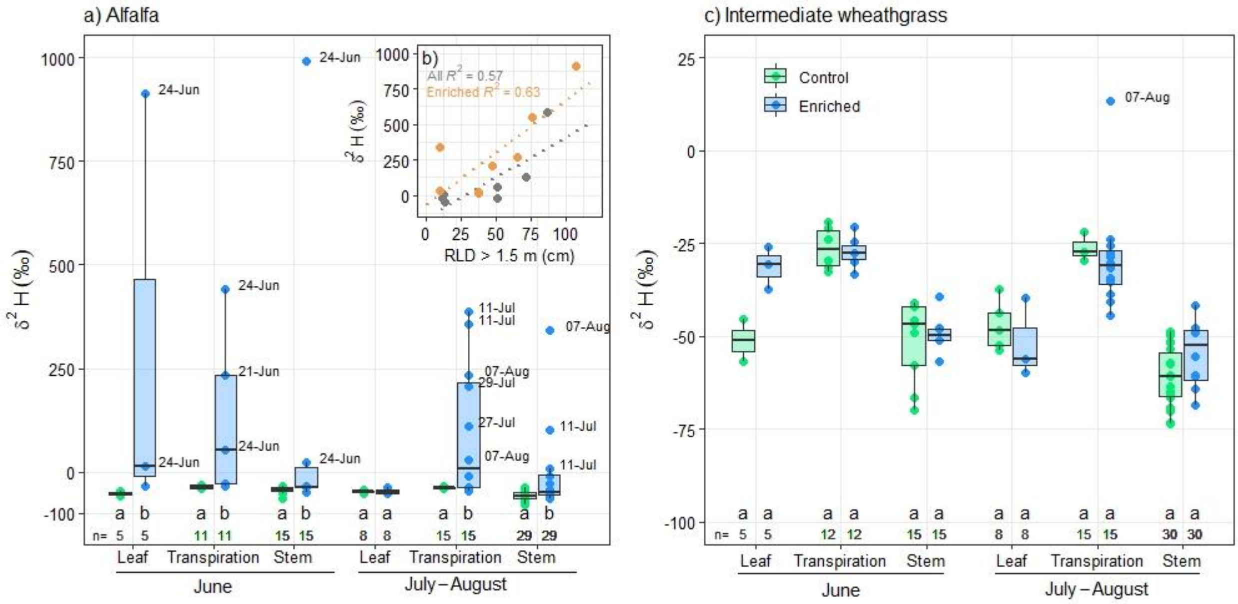


Figure 8. Boxplots and measured values (points) of δ^2H in water originated from leaf, stem and transpiration of alfalfa (a) and intermediate wheatgrass (c). Comparisons were made between control (green) and enriched sites (blue) for each sample type and time period, letters that differ indicate significant differences at ($p<0.05$). Dates of sample collection are provided solely for positive δ^2H values. (b) Relationship between average δ^2H in water samples and total root length found below 1.5 m. The grey line includes all samples collected and the orange line includes only positive δ^2H values samples. Average were calculated for each sample type and sites

Modelling approach

- *Simulation of soil hydraulic parameters and soil water content*

The soil hydraulic parameters adjusted by inverse modelling allowed an effective prediction of the soil water movement for both crops (RMSE= 0.01; $R^2 > 0.60$) under different compensation scenarios (Table 4). This conclusion can be drawn because, first, the fitted soil water retention curves match the measured values well and are similar across simulations (Supplemental Fig. 1). Small differences in soil water retention curves were predicted for the bottom layers which suggest a plot/crop effect. Second, the fitted soil conductivity curves are also similar across simulations (Supplemental Fig. 4). Third, over the two years' time course, the different simulations predicted the soil volumetric water content evolution relatively well: gradual decrease of the soil water in the spring and replenishment of the soil profile in the autumn (Supplemental Fig. 5). However, some discrepancies from these statements are noticeable. In particular, the soil profile was re-wetted earlier in November 2018 for all simulations compared to the measured values (Supplemental Fig. 5). In addition, the worst model predictions were obtained for the simulation of intermediate wheatgrass with a full compensation (ω_c=0.1) as the simulated soil water content at 0.75 m dropped much lower than the measured values (Supplemental Fig. 5) and the soil conductivity curve in the topsoil showed higher values than others simulations (Supplemental Fig.

4). Nevertheless, the soil fitted parameters and simulated values of soil water content closely match the measured values, suggesting that the models accurately described water movement at the soil-plant and field level. Given the limited amount of measured data (i.e. drying cycle at only two soil depths and a single measured retention curve), some degree of variation is expected. Nonetheless, simulations with low compensation levels (i.e. 0.75, 0.875, 1), presented results of root water uptake and plant transpiration within a similar range (Fig. 9; Supplemental Fig. 6). In conclusion, these models are suitable for the purpose of this study, which was to provide an estimate (i.e. range) of the contribution of deep soil layers to the crop transpiration. For simplicity, all simulation results will be presented on a yearly basis and only simulation with a compensation factor of 0.75 will be presented monthly.

Table 4. Goodness of fit of the different models. Root Mean Square Error (RMSE)

Compensation level	Parameter	Alfalfa	Intermediate Wheatgrass
ω_c=0.1	R^2	0.71	0.79
	RMSE	0.01	0.01
ω_c=0.75	R^2	0.69	0.85
	RMSE	0.01	0.01
ω_c=0.875	R^2	0.65	0.82
	RMSE	0.01	0.01
ω_c=0.9	R^2	0.62	0.80
	RMSE	0.01	0.01

- *Root water uptake profiles*

For all simulations, alfalfa showed higher evapotranspiration due to its longer growing season and higher crop factor. Alfalfa used more water from deep soil layers (i.e., >1 m) compared to intermediate wheatgrass (Fig. 9ab). For both crops, actual transpiration was lower than the potential transpiration except for the simulation with a full compensation (i.e. ω_c=0.1) suggesting the crops had been water stressed under all other scenarios. Both crops showed higher water stress in 2018 than 2019. For both crops, the influence of the different compensation factors was noticeable during periods of water stress (i.e. 2018) with increased contribution of the subsoil layers to the plant transpiration. When considering all the scenarios, alfalfa used annually between 68 to 157 mm from the 1.0 - 1.5 m layer covering 20 to 30% of the plant transpiration and between 13 to 53 mm from the 1.5 – 2.0 m layer covering respectively 4 to 10% of the plant transpiration.

In comparison, intermediate wheatgrass had a lower uptake with 28 to 123 mm taken annually from the 1.0 - 1.5 m layer covering 9 to 27% of the plant actual transpiration. From the 1.5 – 2.0 m layer, intermediate wheatgrass used between 4 to 27 mm annually covering 1 to 6% of the plant actual transpiration.

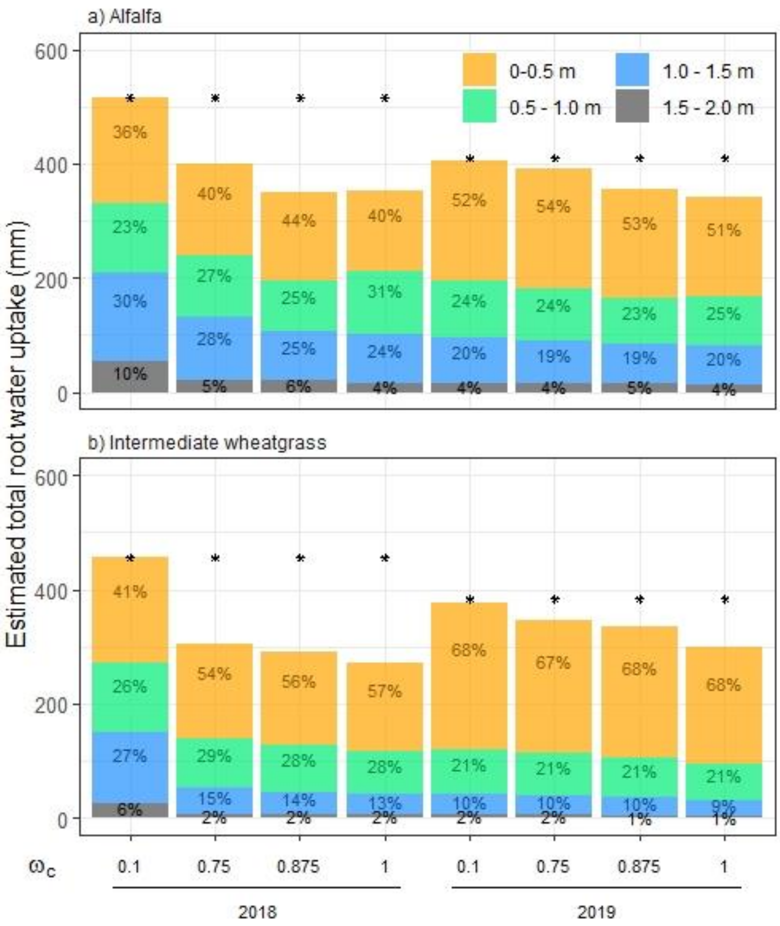


Figure 9. Comparison of different compensation levels (i.e. ω_c= 0.1, 0.75, 0.875, 1) on cumulative root water uptake of alfalfa (a) and intermediate wheatgrass (b) in 2018 and 2019. Actual root water uptake is divided in four root water uptake depths, 0-0.5 m (yellow), 0.5-1.0 m (green), 1.0-1.5 m (light blue) and 1.5-2.0 m (grey). Percentage values indicates the relative contribution of each soil layer. (*) Potential transpiration

When focusing on the scenario with a moderate compensation level (i.e. ω_c=0.75), the monthly water uptake profile differs between the two crops and the two years (Fig. 10ab). For both crops, actual transpiration was lower than the potential transpiration in June, July and August 2018 suggesting that the plants had been particularly water stressed during those months. Under less water-stressed conditions (i.e. 2019) intermediate wheatgrass used more water from the 0-0.5 m soil layer compared to alfalfa. However, during both growing seasons both crops tended to take up similar amounts of water from the 0.5-1.0 soil layer (Fig. 10). For both crops, contribution of deep water increases during the season with a peak around July. In July 2018, alfalfa used approximately 28 mm and 8 mm respectively from the 1.0 to 1.5 m and 1.5 to 2.0 m layers against 16 mm and 4 mm, respectively in 2019. During this particularly dry month, the relative contribution of deep water to plant transpiration was more than doubled in comparison to 2019 and reached 47% and 13% respectively for the 1.0 to 1.5 m and 1.5 to 2.0 m layers. In comparison, in July 2018, intermediate wheatgrass used 12 mm and 3 mm of water from the 1.0 to 1.5 m and 1.5 to 2.0 m layers, respectively against 9 mm and 2 mm in July 2019. However, deep water used from the 1.0 to 1.5 m and 1.5 to 2.0 m layers still contributed up to 34% and 8% of the plant transpiration in July 2018. As for alfalfa, the relative contribution of deep soil layers to plant transpiration was more than doubled in intermediate wheatgrass in 2018 compared to 2019 during months where water stress occurred.

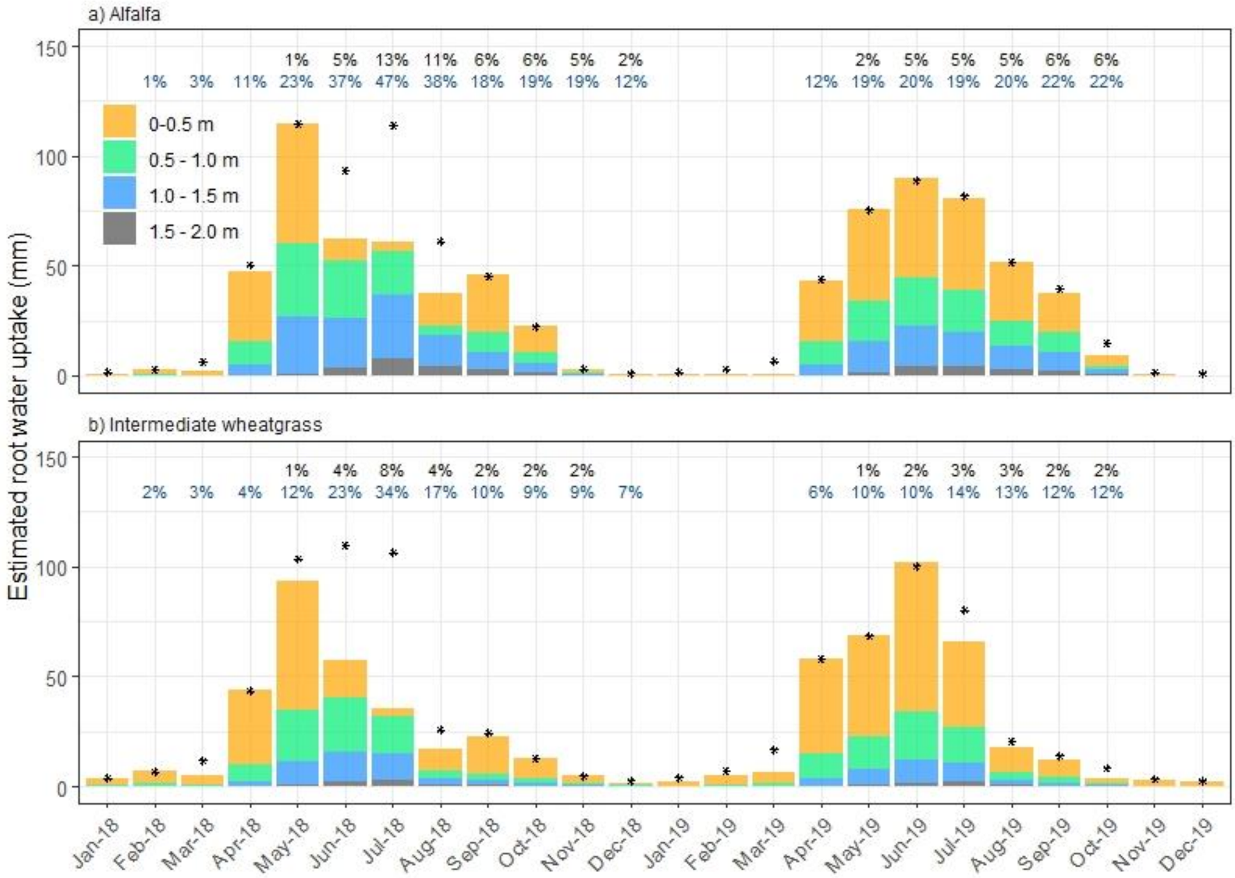


Figure 10. Monthly estimated root water uptake of alfalfa (a) and intermediate wheatgrass (b) over the 2018 and 2019 season. Simulation results for $\omega_c = 0.75$. Actual root water uptake is divided in four root water uptake depths, 0-0.5 m (yellow), 0.5-1.0 m (green), 1.0-1.5 m (light blue) and 1.5-2.0 m (gray). Percentage values indicates the relative contribution of the 1.0-1.5 m and 1.5-2.0 m soil layer, respectively. (*) Potential transpiration

Discussion

In this study, we found that alfalfa was deeper rooted and used more water from deep soil layers than intermediate wheatgrass. Although all methods converged towards this conclusion, differences in root distribution and water uptake were identified depending on the method used. The first part of this discussion focuses on differences observed between the methods while second part will focus on crop differences in terms of root growth and water uptake.

Methodological comparisons

- <u>*Root measurement methods*</u>

The trend in root distribution was quite different between the root measurement methods used and the crops studied. In the topsoil, alfalfa presented greater root length than intermediate wheatgrass with the minirhizotron method but lower root length with the coring method. The coring method showed that root length density of alfalfa decreased with soil depth whereas the minirhizotron method showed peaks in root length within the 0.75-1.75 m soil layer. Wahlström et al. (2015) concluded that such plant responses are partly due to preferential root growth and increased branching that could occur along the minirhizotron tube. Poor tube/soil contact in the upper part of the tube could also explain the discrepancies identified in the topsoil (Merrill et al. 2004).

- *Stable isotope experiment*

In all plant water samples, natural abundance of water isotopes were more enriched than any of the soil layers (Fig. 7) which prevented the use of a mixing model to estimate the average depth of water absorption by the roots. Enrichment of water at the plant level could, among others, originate from (1) fractionation of water in the leaf during transpiration (Dawson and Ehleringer 1993), (2) exchange of water from the phloem to the xylem (Farquhar et al. 2007) and (3) mixing of water with different residence time and isotopic signature (Rothfuss and Javaux 2017a). Analysis of the slope of the $\delta^{18}O$-$\delta^{2}H$ regression line against the LMWL indicated that water samples in the leaf were more enriched water followed by the stem and the transpiration samples. The smaller difference in slope obtained between the LMW and the transpiration line was surprising but is most likely due to the equilibrium fractionation that occurs during both transpiration and condensation of the water.

The correlation between root length density and the soil isotopic signature suggested that roots, by modifying the soil water cycle, influence the soil isotopic signature. At the study site, rainfall has different isotope signatures depending on the season and therefore deeper soil layers will be replenished with water having a different isotope signature than shallower ones (Gazis and Feng 2004; Nielsen et al. 2010). Roots, by altering the water cycle in their surrounding soil layer, are changing the isotopic signature of the soil by allowing its replenishment from rainfall of a different isotopic signature. The enrichment in heavy isotope in the topsoil was most likely due to equilibrium fractionation that occurs during evaporation and replenishment of the topsoil layer by enriched summer rainfall. In subsoil layers, it is supposed that higher root length density will remove substantial amount of soil water and allow replenishment of the soil layers with more depleted autumn and winter rainfalls as illustrated by the soil moisture patterns (Fig. 4).

Results obtained from the tracer labelling study suggest a much bigger difference in root water uptake at 2.0 m between alfalfa and intermediate wheatgrass than results obtained with TDR sensors and simulation work. One explanation for this difference could be that the soil conditions at the time of injection have a great influence on the tracer movement. At the time of injection the 2.0 m soil layer under alfalfa was drier than under intermediate wheatgrass (Supplemental Fig. 3a). In addition, the groundwater level was also lower in the alfalfa plot than in the intermediate wheatgrass plot (Fig. 4c). For intermediate wheatgrass, such wetter conditions could have favored the diffusion and dilution of the tracer solution within the soil water and limited root activity due to anoxic conditions.

- *Soil moisture content monitoring and simulation of water fluxes*

Both TDR sensors and the simulation study showed an increase in deep water uptake throughout the season. However, Hydrus-1D predicted higher deep water uptake in 2018 than in 2019 whereas TDR sensor measurements indicated the opposite trend with support from the minirhizotron data. Indeed, sensors measurements showed that in 2019, soil water depletion at 0.75 and 1.5 m depth occurred one month earlier for both crops and that alfalfa depleted the soil profile to a greater extent (i.e. 2.5 m), than in 2018. This suggests that crops were able to use deep soil water earlier and to a larger extent in 2019 with smaller differences observed for intermediate wheatgrass. This conclusion is supported by minirhizotron measurements which showed that alfalfa had greater root length at depth in 2019 than in 2018. Root length distribution at depth was relatively similar between the two years for intermediate wheatgrass, supporting the fact that smaller differences in soil water depletion were observed. In this simulation study, main limitation comes from that fact that the model uses a fixed root distribution along the profile to derive root water uptake, which disregards the dynamics of root growth (Vadez 2014). In our case, using a fixed root distribution for both years based on measurements done in 2019 constitutes a non-realistic assumption that restricts our simulation based conclusion on root water uptake in 2018. In consequence, model simulations likely overestimate the capacity of crops to uptake water from deep soil layers in 2018 due to greater root length at depth in 2019. However, this approach is still relevant to estimate the contribution of deep subsoil layers to the plant transpiration and to study the role that root length at depth could play under drought conditions. Further differences between the Hydrus-1D and the TDR sensors readings could also be due to (1) root length density in the vicinity of the sensors, (2) water infiltration and capillary rise in the soil and (3) the soil structure around the sensor. Nonetheless, simulated soil water retention and conductivity curves and soil water content were well correlated to measured observations at specific depths, which allow us to conclude that this model provides relevant results on deep root water uptake of these two perennial crops.

Empirical evidence of deep roots and deep water uptake

- *Crop performance and stress*

Harvested biomass, TKW, stomatal conductance and simulation results indicated that both crops experienced severe water stress in 2018. For intermediate wheatgrass, the dry spring of 2018 restricted vegetative development before the reproductive phase which favoured the formation of fewer but larger grains compared to 2019 (Korte and Chu 1983; Blum et al. 1990). In perennial grasses, grain yield varies over years and usually peaks during the 2nd or 3rd years and then declines

(Wagoner 1990). High variability in intermediate wheatgrass grain yields is found in the literature with second year yield ranging between 0.4 and 1.7 t/ha depending on climate, environmental conditions, farming management and selection lines (Culman et al. 2013; Pugliese et al. 2019). While our second- and third year yield fall within this range, it is possible that a yield reduction occurred in 2018 and was masked by the difference in yield potential between years. For alfalfa, yield from the June harvest was lower in 2018 suggesting an effect of the dry 2018 spring. Harvested biomass of the two later cuts were very similar and annual forage yields are in line with the yield range found in the literature (i.e. 10 - 20 t/ha) (Riday and Brummer 2002).

- *Rooting profile*

We found that both crops developed fine roots deeper than 2.0 m as found in other studies (Dardanelli et al. 1997; Ward et al. 2002; Cox et al. 2006). Overall, alfalfa was deeper rooted and presented a higher proportion of roots at depth compared to intermediate wheatgrass.

For intermediate wheatgrass, root length was highest in the topsoil (i.e. >0.5 m) and root density decreased steeply with soil depth as observed in others studies (Sprunger et al. 2018; Pugliese et al. 2019). Minirhizotron measurements showed that intermediate wheatgrass presented lower root length in the topsoil layers in May 2019, most likely in response to the very dry April month during that year. Apart from this, no clear difference in rooting profile was observed between the two years, suggesting that the drought of 2018 had little effect on root growth. This lack of differences between the two years also suggests a high rate of root turnover. Either new roots are established each year, most likely from newly formed tillers, or water-saturated conditions in winter lead to root die back (Herzog et al. 2016). In fact, it is still unclear which portion of the root system of intermediate wheatgrass remains active over time and especially after harvest, which should require particular attention (Cox et al. 2006). Nevertheless, the coring method reinforced the idea that intermediate wheatgrass had interesting characteristics in terms of deep rooting with higher root length density below 1.5 m soil depth than previously reported for spring and winter wheat (Gregory et al. 1979; Kirkegaard et al. 2007).

With more than 20% of the plant total root length below 1.0 m, alfalfa had deeper and more evenly distributed roots throughout the soil profile than intermediate wheatgrass. For alfalfa, peaks in root length identified within the 0.75-1.75 m soil layer along minirhizotron tubes suggest that roots grew preferentially at depth and that over time alfalfa gradually builds a deeper root system (Pietola and Smucker 1995). The fact that alfalfa presented lower root length along minirhizotron tubes in 2018 in comparison to 2019, suggests that root grow deeper from older roots or/and that root growth was hampered by the particular dry condition of that year (Sheaffer et al. 1988; Erice et al. 2010). We did not find roots deeper than 3 m for alfalfa, as reported by Borg and Grimes (1986). In fact, at this location, root growth of both crops was most likely hampered by the high soil bulk density encountered in the subsoil and saturated water conditions during part of the winter. Tap-rooted species like alfalfa have a higher penetration capacity of highly compacted soil layers due to greater root diameter when compared to fibrous rooted species like intermediate wheatgrass (Materechera et al. 1992). Dicotyledonous crops, such as alfalfa, may also have a better tolerance to waterlogging conditions than monocotyledonous crops, such as intermediate wheatgrass, due to secondary thickening of their root tissue which reduces oxygen loss, as hypothesized by Strock and Lynch (2020). Both crops had a deeper root system in 2019, when the groundwater level was lower.

- Root water uptake

All methodological approaches indicated that alfalfa was taking up more water from deep soil layers than intermediate wheatgrass and allowed us to conclude that both crops were capable of taking water down to 2 m soil depth. Overall, model simulations showed that alfalfa took approximately twice as much water as intermediate wheatgrass below 1.0 m soil depth. Crop differences in deep water uptake are likely to be due to differences in (1) root distribution, (2) root hydraulic conductivity at depth and (3) crop water demand. Firstly, the spatial distribution of roots in the soil influences the development of the soil water potential and of the plant response (e.g. ABA) (Draye et al. 2010). In fine textured soil like in this study, high root length density in the topsoil will induce a rapid drop of the soil water potential and thus force intermediate wheatgrass to rely on fewer roots at depth. In contrast, alfalfa with a less tapered root distribution might present a more homogeneous water uptake throughout its root zone. Secondly, alfalfa presented a larger amount of coarse roots at depth compared to intermediate wheatgrass. Coarse roots with more abundant metaxylem vessels will have a higher axial hydraulic conductance favouring plant water uptake from deeper soil layers compared to cereals with finer roots (Hamblin and Tennant 1987; Zwieniecki et al. 2003, Chapter 3). Finally, alfalfa presented a higher stomatal conductance and transpiration, especially because transpiration of cereals decreases during the reproductive stage (Allen and Pereira 1998). Considering that alfalfa is known to extensively deplete the soil water profile, down to 10 m depth in some cases (Li and Huang 2008), it is likely that, in this study, rooting depth and water uptake of alfalfa were limited by soil constraints and climatic conditions. Intermediate wheatgrass is known for taking up more water from the 0.5-1.0 m soil layer than annual wheat (Culman et al. 2013; Sprunger et al. 2018) and other perennial grasses (Sainju et al. 2017). Our results concord with such findings as intermediate wheatgrass was as efficient as alfalfa in taking up water from the first meter of soil and even more efficient in the topsoil layers (i.e. <0.25 m). Furthermore, our simulation results showed interesting water uptake after anthesis for intermediate wheatgrass (i.e. 30 and 22 mm from the 1.0 m – 2.0 m layer in 2018 and 2019, respectively).

During the 2019 season, TDR sensor measurements showed that both crops used water from deep soil layers earlier than in 2018. In addition, alfalfa induced changes in soil moisture content down to a depth of 2.5 m in 2019. These empirical measurements suggest that alfalfa used more water from deep soil layers in 2019 compared to 2018. In contrast, they suggest that intermediate wheatgrass used similar amounts of water from deep soil layers in both years. Simulation results showed that for both crops, deep water uptake increased from April to July. This increase occurred during critical stages and during the driest month of the growing season which makes it of great importance to maintain yield under dry conditions (Kirkegaard et al. 2007; Lilley and Kirkegaard 2011). Assuming equal root length at depth in 2018 and 2019, model simulations predicted that deep water use substantially increased under drought for both crops. While such results were obtained with a moderate compensation factor (i.e. ω_c=0.75), higher water uptake from deep soil layers in 2018 was still predicted under the non-compensation scenario (ω_c=1; Fig. 9). These findings highlight the role that root length at depth plays in mitigating drought impact. This conclusion is supported by the correlation of deep root length to the uptake of tracer in the labelling experiment. However, the fact that we found (1) little tracer uptake for intermediate wheatgrass and (2) no increase in tracer uptake in spite of the increase in tracer application during the July/August period, suggest that root length is not the sole driver behind water uptake (Hamblin and Tennant 1987). In particular, deep root water uptake depends on the crop water demand, hydraulic conductivity and compensation effects that occur at the plant level (Jarvis 1989; Skaggs et al. 2006; Simunek and Hopmans 2009). However, estimating the plant hydraulic conductivity and the extent to which compensation occurs at the plant level remain highly challenging when modelling root water uptake (Cai et al. 2017). Under a scenario of nearly full compensation (ω_c = 0.1) plants did not experience water stress. However, such a scenario does not match with our crop measurements and model fit was lower under this scenario. As speculated by Simunek and Hopmans (2009), it is more likely that herbaceous plants have lower compensation levels of around ω_c=0.75 which is in line with results from (Cai et al. 2017) on winter wheat. Although passive compensation had certainly taken place through redistribution of hydraulic pressure within the plant, our data do not support the idea of active compensation under drought (i.e. development of roots in wet soil layers) as reported for other crops like chicory (Vandoorne et al. 2012).

Conclusion

In this study intermediate wheatgrass and alfalfa used water from deep soil layers (i.e. 2.0 m) due to their extensive root system. Intermediate wheatgrass had a lower proportion of roots at depth in comparison to alfalfa. Results from TDR sensor measurements and the deuterium tracer labelling experiment showed that intermediate wheatgrass used less water from deep soil layers than alfalfa. Model simulations predicted that alfalfa used twice as much water below 1.0 m soil depth than intermediate wheatgrass. Nonetheless, intermediate wheatgrass was predicted to use more than 20 mm of water from below 1 m soil depth after anthesis. Crop differences in water uptake capacity were most likely due to differences in crop water demand and root distribution.

Both crops were severely water stressed in 2018 in comparison to 2019. TDR sensors and minirhizotron measurements showed that alfalfa was deeper rooted and used more water at depth in 2019 than in 2018. For intermediate wheatgrass, no clear differences were observed between the two years. Assuming, equal root length at depth in 2018 and 2019, the simulation study predicted that deep water uptake would substantially increase during the 2018 drought. The deuterium tracer experiment further supports this observation as tracer uptake was correlated to root length density at depth in the case of alfalfa. Therefore, deep rooting was found beneficial under topsoil drying and in the event of drought. Intermediate wheatgrass presents interesting potential to exploit deep soil water and both crops have high potential in regions with high soil water availability and significant groundwater recharge.

In the case of alfalfa, results from tracer labelling experiment showed higher differences in root water uptake at 2.0 m soil depth than predicted by the TDR sensors and simulation study. Root length measurement with the minirhizotron method also showed higher root growth at depth than the soil coring method for alfalfa. These results demonstrate that the application of common root research methods in long-term field experiments is challenging due to the environmental variability and plant adaptations to it. Combination of several experimental methods allows a better understanding of this variability and for more robust conclusions to be drawn.

Supplementary material

Supplementary tables

Supplemental Table 1. Soil, transpiration water and leaf/stem date of sampling

Day from labelling		0	+1	+4	+8	+24	-3	0	+2	+4	+8	+16
Date		17-06	18-06	21-06	24-06	11-07	18-07	22-07	24-07	27-07	29-07	07-08
Tracer study	Transpiration	**Labelling**		**x**	**x**	**x**		**Labelling**		**x**	**x**	**x**
	Leaf/Stem			**x**	**x**	**x**				**x**	**x**	**x**
Natural abundance	Transpiration			**x**	**x**				**x**			
	Leaf/Stem		**x**	**x**	**x**	**x**			**x**	**x**	**x**	**x**
	Soil						**x**					

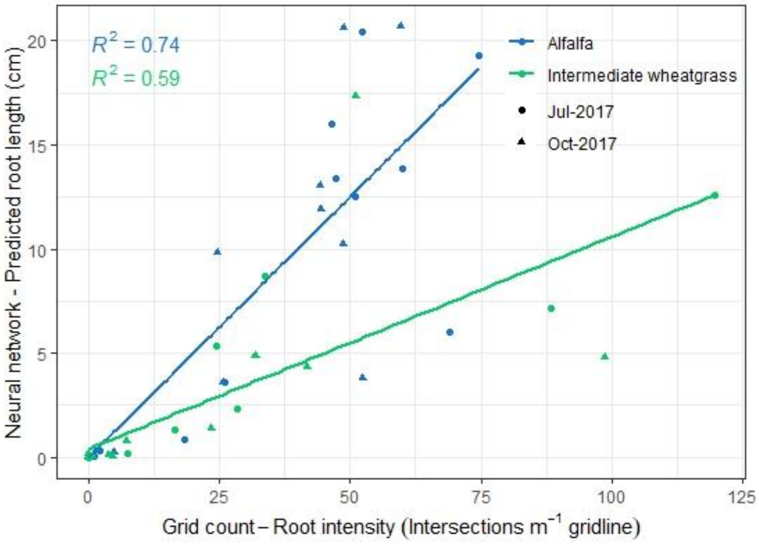
Supplemental Table 2. Alfalfa and intermediate wheatgrass morphological stages and height. Average value with standard error in brackets

	Alfalfa		Intermediate wheatgrass	
Date	Phenological stage	Mean (se)	Phenological stage	Mean (se)
27-05-18	Early flower	0.90 (0.03)	(R2) Spikelet fully emerged	1.04 (0.02)
18-06-18	Early seed pod	1.04 (0.04)	(R3) Inflorescence emerged	1.53 (0.04)
05-07-18	Mid-vegetative	0.26 (0.03)	(S2) Soft dough	1.44 (0.03)
20-07-18	Late flower bud	0.56 (0.02)	(S2) Soft dough	1.58 (0.05)
07-08-18	Late seed pod	0.66 (0.03)	(S5) Dry seed ripe	1.54 (0.03)
04-09-18	Early vegetative	0.36 (0.03)	(Vn) Nth leaf collared	0.44 (0.02)
28-09-18	Late vegetative	0.57 (0.03)	(En) Node palpable/visible	0.37 (0.02)
25-10-18	Late vegetative	0.66 (0.02)	(En) Node palpable/visible	0.40 (0.02)
30-04-19	Mid vegetative	0.37 (0.01)	(En) Node palpable/visible	0.34 (0.02)
13-05-19	Late vegetative	0.53 (0.02)	(En) Node palpable/visible	0.45 (0.01)
29-05-19	Early Flower buds	0.80 (0.02)	(En) Node palpable/visible	0.76 (0.03)
07-06-19	Early Flower buds	0.96 (0.03)	(R1) Inflorescence emergence	1.09 (0.03)
24-06-19	Late flower	1.12 (0.05)	(R3) Inflorescence emerged	1.65 (0.03)
10-07-19	Mid vegetative	0.29 (0.01)	(S1) Milk	1.70 (0.03)
24-07-19	Early flower	0.66 (0.02)	(S2) Soft dough	1.67 (0.03)
07-08-19	Later flower	0.84 (0.02)	(S4) Endosperme hard	1.61 (0.03)
03-10-19	Late vegetative	0.64 (0.01)	(Vn) Nth leaf collared	0.26 (0.02)
21-10-19	Late vegetative	0.66 (0.02)	(Vn) Nth leaf collared	0.23 (0.02)

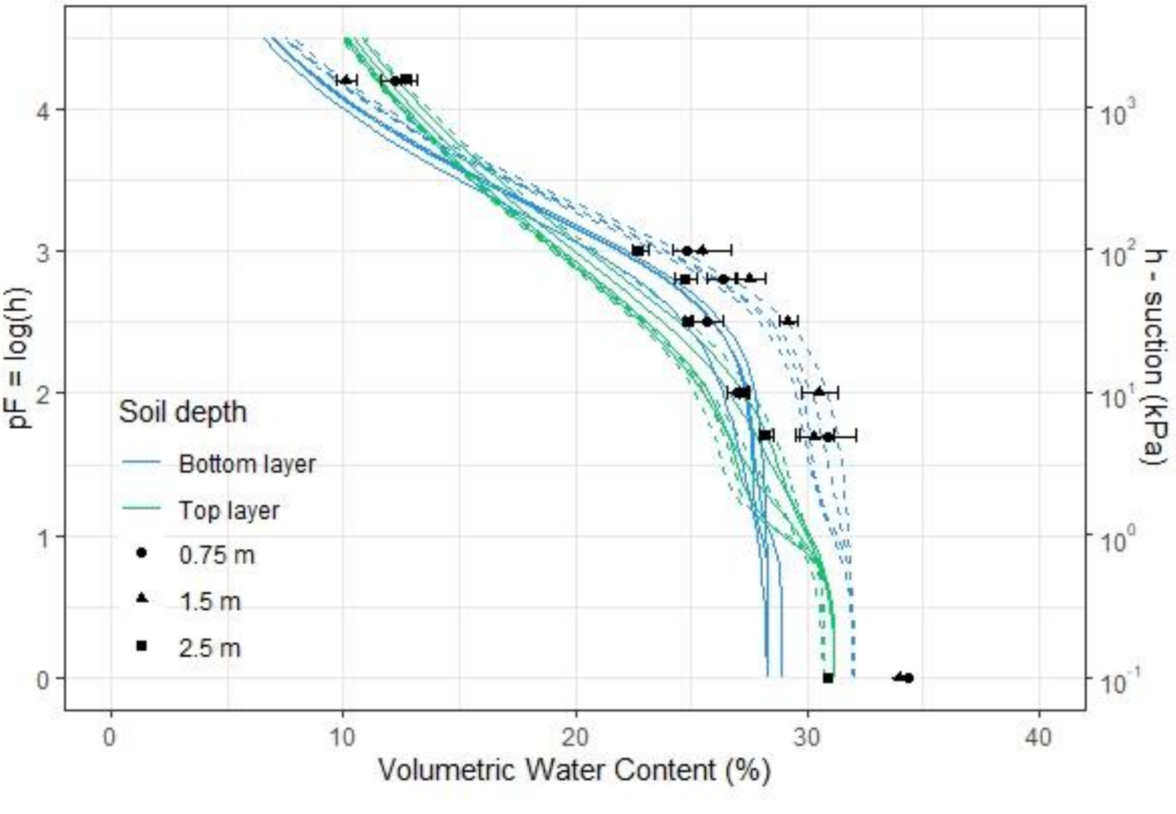
Supplemental Table 3. Adjusted soil hydraulic parameters of the different models (i.e. ω_c=0.1, ω_c=0.875 and ω_c=1.0). All parameters presented here have been estimated by inverse modeling and standard errors are provided. Fixed parameters can be found in table 2

Compensation factors	Parameter	Unit	Alfalfa	Intermediate Wheatgrass
ω_c=0.1	1-Ks	cm/sec	2.89±0.2	3.70±1.0
	1-L	-	0.16±1.4e^{-2}	0.22±0.1
	1-W$_f$	-	0.07±4.1e^{-3}	0.04±3.7e^{-3}
	1-α_f	cm^{-1}	0.14±9.1e^{-3}	0.11±1.7e^{-2}
	1-n$_f$	-	2.45±1.2e^{-1}	2.83±0.4
	2-Ks	cm/sec	2.84±1.9e^{-2}	1.64±0.4
	2-L	-	10.00±0.9	3.71±0.4
	2-W$_f$	-	0.04±2.0e^{-3}	0.05±2.8e^{-3}
	2-α_f	cm^{-1}	0.10±7.1e^{-3}	0.10±1.5e^{-2}
	2-n$_f$	-	3.83±0.4	1.22±7.7e^{-2}
ω_c=0.875	1-Ks	cm/sec	4.09±0.8	0.33±8.8e^{-3}
	1-L	-	0.61±0.7	1.9e^{-4}±4.7e^{-5}
	1-W$_f$	-	0.10±8.7e^{-3}	0.13±2.3e^{-3}
	1-α_f	cm^{-1}	0.10±7.0e^{-3}	0.09±2.1e^{-3}
	1-n$_f$	-	2.05±0.3	5.28±5.4e^{-2}
	2-Ks	cm/sec	0.17±2.0e^{-2}	2.36±1.1e^{-2}
	2-L	-	10.00±1.6	1.30±1.3
	2-W$_f$	-	3.8e^{-3}±5.4e-3	0.05±3.2e^{-4}
	2-α_f	cm^{-1}	0.11±0.2	0.10±1.2e^{-3}
	2-n$_f$	-	3.08±17.83	3.29±2.4e^{-2}
ω_c=1.0	1-Ks	cm/sec	2.06±0.2	0.30±5.2e^{-4}
	1-L	-	0.76±0.9	0.01±1.9e^{-4}
	1-W$_f$	-	0.13±5.8e^{-3}	0.12±1.9e^{-3}
	1-α_f	cm^{-1}	0.10±9.4e^{-3}	0.10±2.3e^{-3}
	1-n$_f$	-	2.33±0.8	1.90±7.2e^{-3}
	2-Ks	cm/sec	8.92±2.3	2.13±4.6e^{-2}
	2-L	-	5.11±0.8	0.01±9.1e^{-5}
	2-W$_f$	-	0.10±5.1e^{-2}	0.07±7.5e^{-4}
	2-α_f	cm^{-1}	0.10±3.9e^{-2}	0.12±1.1e^{-3}
	2-n$_f$	-	1.32±0.1	2.34±6.6e^{-2}

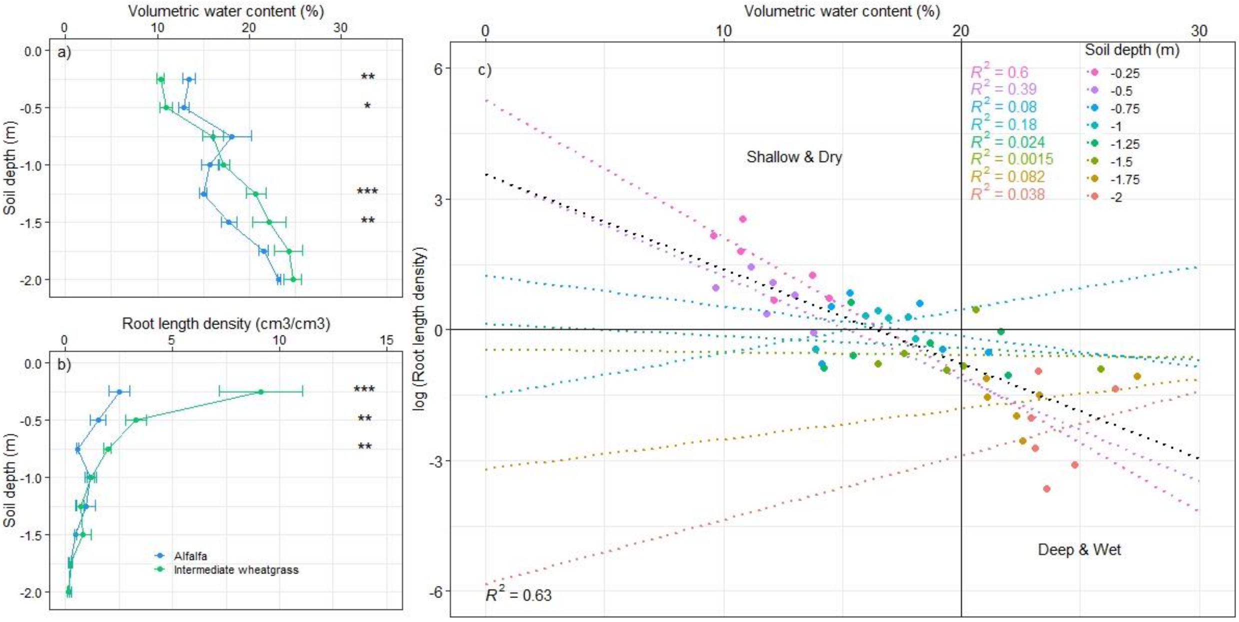
Supplementary figures



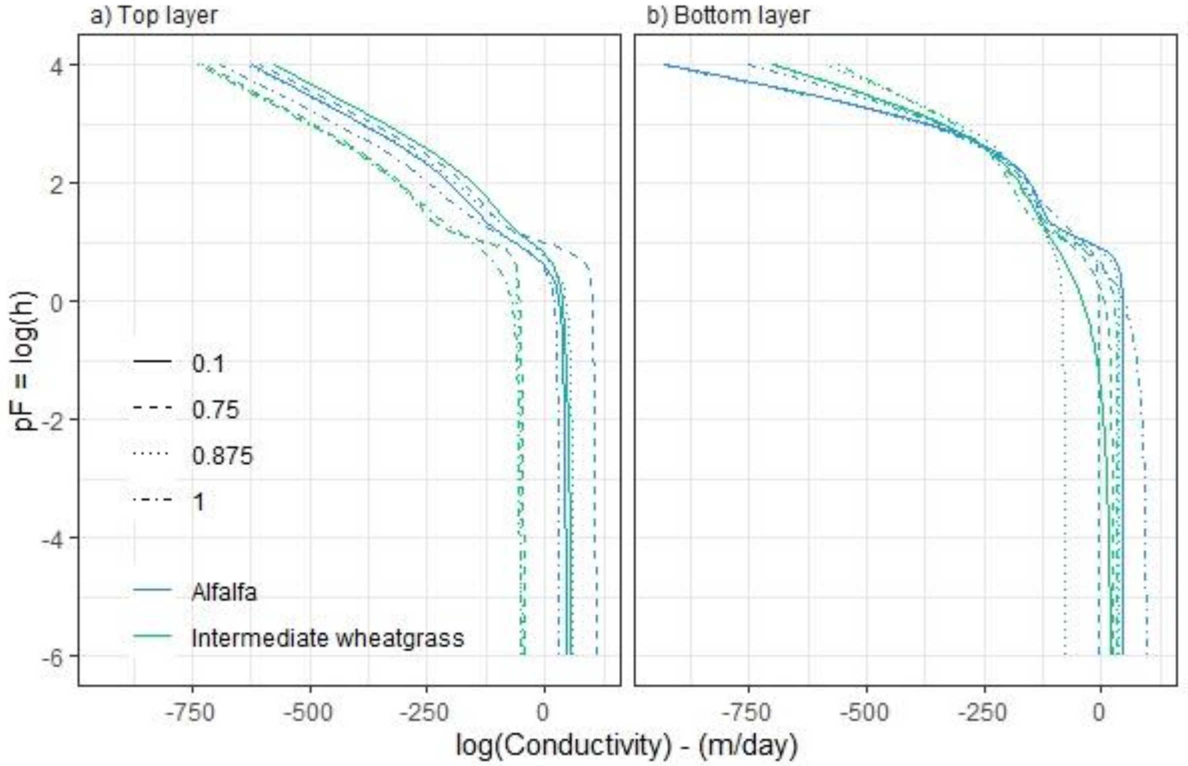
Supplemental Figure 1. Correlation between average root intensity and average segmented root length for alfalfa and intermediate wheatgrass. Averages represents 0.25 m soil depth intervals. A total of 2854 images were segmented and counted for this test



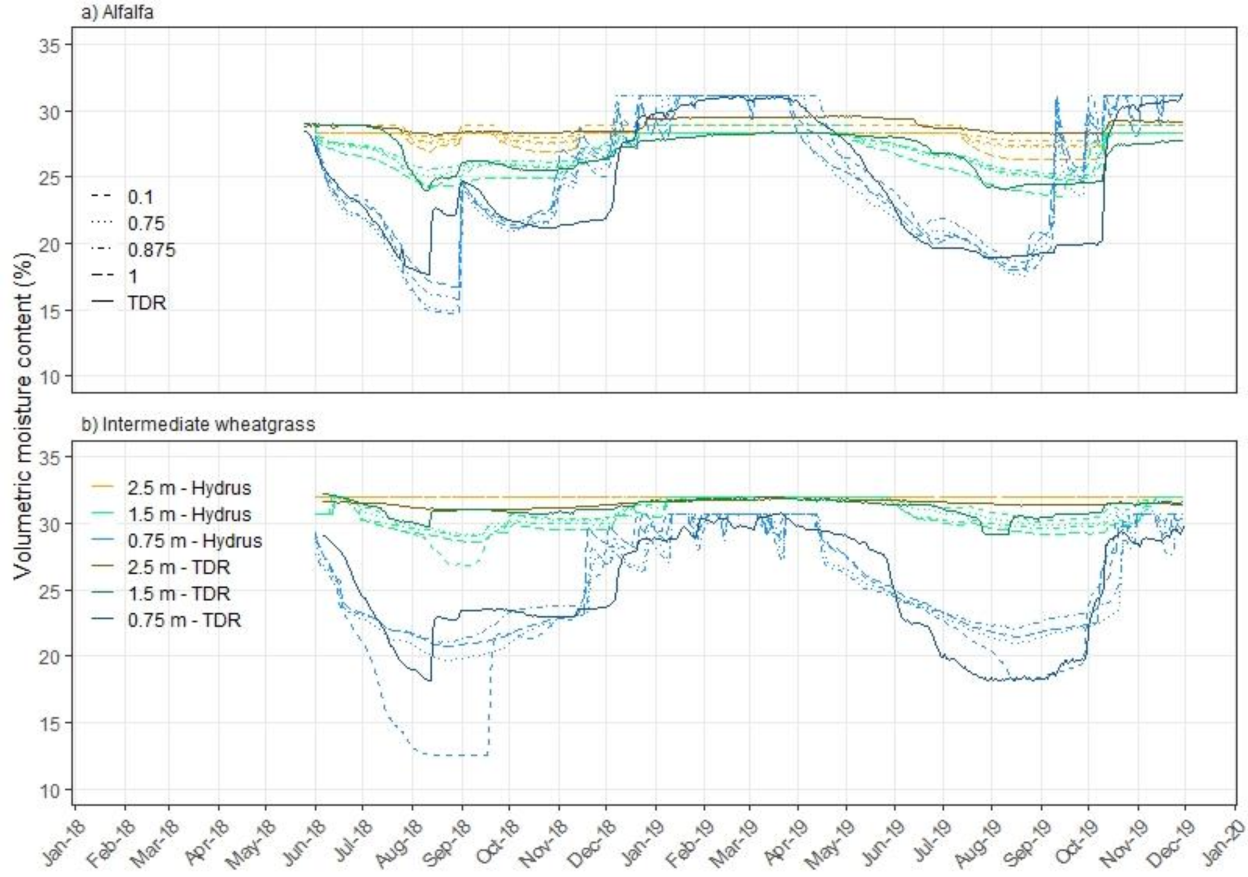
Supplemental Figure 2. Soil water retention curve measured at 0.75, 1.5 and 2.5 m soil depth (points - average value ± standard error) and simulated for the top (Green) and bottom (Blue) layer in Hydrus-1D and for alfalfa (dashed line) and intermediate wheatgrass (solid line). Different lines represent simulations with different compensation level



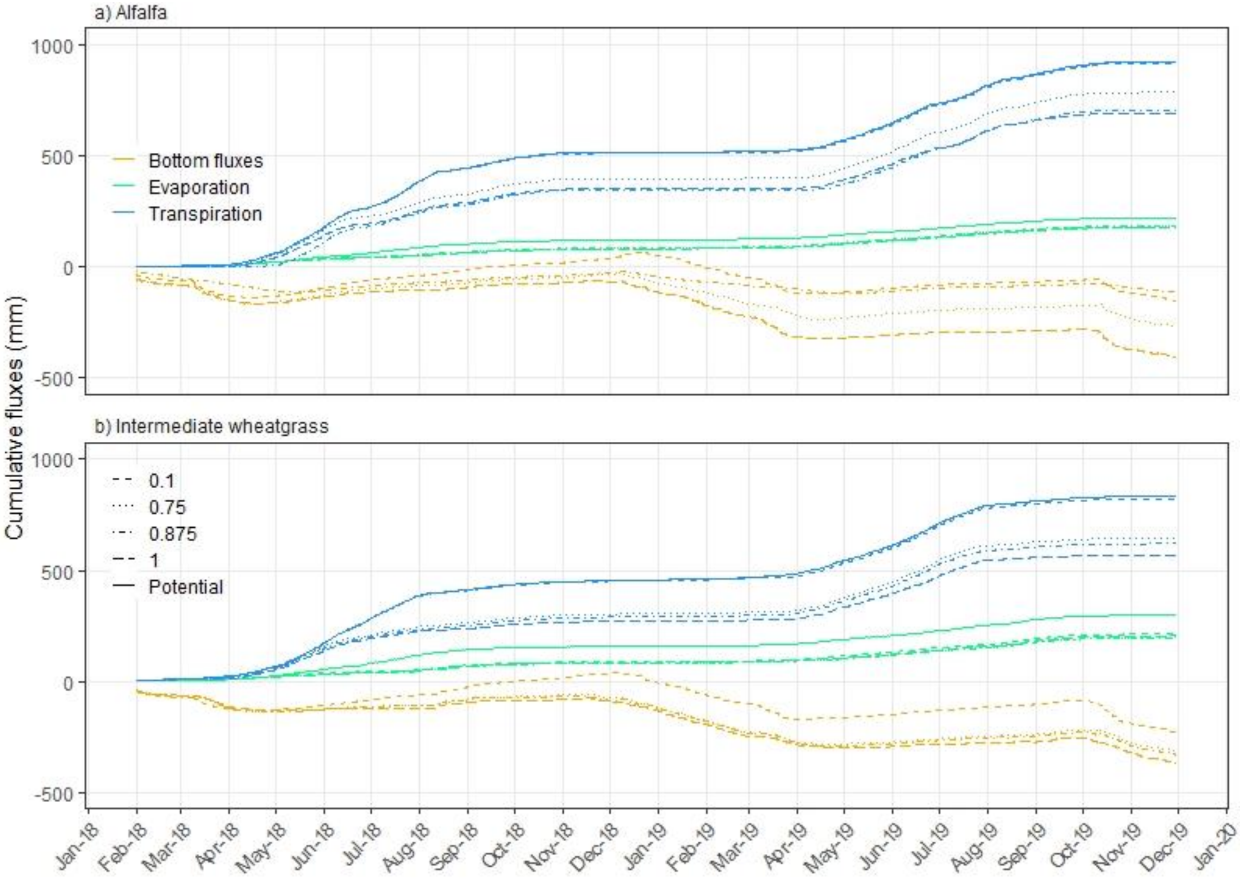
Supplemental Figure 3. Soil volumetric water content (a) and root length density (b) evolution with soil depth. Average value ± standard error. Sampled on 27th of July 2019. Correlation between root length density (log) and soil water content (c). Overall correlation is represented in black



Supplemental Figure 4. Simulated soil hydraulic conductivity for the top (a) and bottom (b) layer in Hydrus-1D and for alfalfa (blue) and intermediate wheatgrass (green). Different lines represent simulations with different compensation level



Supplemental Figure 5. Simulated (dashed line - Hydrus) and measured (solid line - TDR) soil volumetric water content evolution at 0.75 m (blue), 1.5 m (green) and 2.5 m (brown) soil depth under alfalfa (a) and intermediate wheatgrass (b). Data covers two growing seasons from 01-05-2018 until the 30-11-2019



Supplemental Figure 6. Comparisons between cumulative evaporation (green), transpiration (blue) and bottom fluxes simulated by Hydrus-1D over the whole study period under alfalfa (a) and intermediate wheatgrass (b). Potential values (solid line) and actual values obtained for different compensation level (dashed line)

Supplementary equations

Hydrus-1D Model functioning and equations

HYDRUS-1D numerically solves the Richards equation (Eq.3) under variable soil water conditions with a sink term that represents plant water uptake.

$$(3)\qquad \frac{\partial\theta}{\partial t}=\frac{\partial}{\partial z}\left[K(h).\ \frac{\partial h}{\partial z}-K(h)\right]+S_a(h)$$

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). For the numerical solution of equation (3), the soil hydraulic properties have been defined using the bimodel van-Genuchten model (Durner 1994). Durner (1994) divided the porous space into two (or more) overlapping regions and used for each region a van Genuchten-Mualem type function (Van Genuchten 1980). In that perspective water retention curve is given by:

$$(4)\qquad \theta(h)=\theta_r+(\theta_s-\theta_r)Se_{total}$$

where θ_s (cm^{3} cm^{-3}) is the saturated water content, θ_r (cm^{3} cm^{-3}) residual water content and Se_{total} (-) is the effective soil saturation (from 0 to 1) given by:

$$(5)\qquad Se_{total}=Se_m+Se_f$$

Where Se_m (-) is the matrix effective saturation and Se_f (-) is the fracture effective saturation given by:

$$(6)\qquad Se_f=w_f\left[1+\left(-\alpha_f h\right)^{n_f}\right]^{-m_f}$$

And

$$(7)\qquad Se_m=(1-w_f)[1+(-\alpha_m h)^{n_m}]^{-m_m}$$

where w_f (-) the ratio of fracture volume to the total soil volume, and finally $\alpha\_i,$ n_i and m_i=1-1/n_i, are shape parameters and index i corresponds to the matrix (m) and fracture (f) regions, respectively. The hydraulic conductivity is given by:

$$(8)\qquad K(Se_{total})$$
$$=K_s\frac{\left(w_f Se_f+(1-w_f)Se_m\right)^L\left(w_f\alpha_f\left[1-\left(Se_f{}^{-m_f}\right)^{-m_f}\right]+(1-w_f)\alpha_m[1-(Se_m{}^{-m_m})^{-m_m}]\right)}{\left(w_f\alpha_f+(1-w_f)\alpha_m\right)}$$

The sink term (S_a) (cm^{3} cm^{-3} day^{-1}) in eq. 2, defined as the plant removal of water from the soil per unit of time, can be described as

$$(9)\qquad S_a(z,h)=\ \alpha(h).Sp(z)$$

where Sp (cm^{3} cm^{-3} day^{-1}) is the potential plant water uptake rate and α a dimensionless ($0 \leq \alpha \leq 1$) function of plant response to water stress for a given pressure head (h) as defined by (Feddes and Raats 2004). Root water uptake is thus optimal at α(h) equal to 1 and reduced to 0 under saturated (anoxic) or dry conditions. Potential uptake is a linear function of the potential transpiration Tp (cm day^{-1}):

$$(10) \qquad S_p(z) = \frac{\beta(z)}{\int_0^{Zr} \beta(z)dz} T_p$$

where $\beta(z)$ (-) is any measured or modelled root distribution function and Zr the length of the root zone. Combining equations (9) and (10) yields the actual transpiration rate Ta (cm.day^{-1}) over the entire root zone:

$$(11) \qquad Ta = Tp \int_0^{Zr} \alpha[h(z)] \ . \ \beta(z)dz$$

A second threshold response function is used to reflect compensatory water uptake that occurs under topsoil drying (Jarvis 1989). To formulate a compensatory uptake we take equation 11 and define ω as:

$$(12) \qquad \omega = \frac{Ta}{Tp} = \int_0^{Zr} \alpha[h(z)] \ . \ \beta(z)dz$$

ω is called the weighted stress index and provides a measure of the total plant stress with value of 1 indicated no stress throughout the root zone. Thus, setting a compensatory uptake threshold ω_C ($0 < \omega_C \leq 1$), transpiration occurs at potential rate when $\omega > \omega_C$ and is increased throughout the root zone by a factor 1/ω when $\omega < \omega_C$ as summarized in equation (13) and (14).

$$(13) \qquad \frac{Ta}{Tp} = 1, \qquad \omega_c < \omega \leq 1 \qquad\qquad (14) \qquad \frac{Ta}{Tp} = \frac{\omega}{\omega_c}, \qquad \omega < \omega_c$$

Upper boundary conditions and fluxes are calculated based on the atmospheric and soil pressure head near the surface. Surface fluxes (i.e. evaporation or infiltration) are calculated with equation (15):

$$(15) \qquad \left| -K\frac{\partial h}{\partial x} - K \right| \leq E \qquad at\ x = \theta t \qquad with\ h_A \leq h \leq h_S$$

Where E is the maximum potential evaporation or infiltration under current atmospheric conditions (θt) with h_A and h_S being respectively the minimum and maximum pressure head allowed under current conditions at the soil surface. h_S is set to 0. h_A is calculated using equation (16):

$$(16) \qquad h_A = \frac{Rt}{Mg} Ln(Hr)$$

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 Hr the relative humidity.

General discussion

This work brings interesting results for both the research and agricultural worlds. The main results of this study are that (1) both crops developed a deep root system and took up water from great soil depth (i.e. 2.0 m), (2) alfalfa used more water from deep soil layers than intermediate wheatgrass, (3) crop capacity to take up water from deep soil layers seems related to root length density at depth, crop water demand and to internal anatomical traits, especially the root axial hydraulic conductivity, (4) deep water uptake increased during the spring-summer season and was predicted to increase under drought, (5) the growth environment had a significant influence on the plant functioning and (6) the quantification of root growth and water uptake varies depending on the research method used.

Crop difference in root growth and deep water uptake

Both crops were found to be deep rooted and presented active roots down to 2.0 m soil depth. Alfalfa had higher root length at depth and fewer roots in the topsoil. In contrast, intermediate wheatgrass had a greater amount of roots in the topsoil and a smaller, but remarkable, amount of roots in the subsoil than alfalfa. Alfalfa used more water from deep soil layers than intermediate wheatgrass. However, during both seasons intermediate wheatgrass used more than 20 mm of water after anthesis from below 1 m soil depth. Deep water uptake, that occur late in the season when the topsoil is dry is highly valuable and is known for resulting in a high water use efficiency (Kirkegaard et al. 2007), which is very promising for the development of a perennial grain crop with improved drought resistance (Passioura 1983). At the root level, deep water uptake also depends on root axial hydraulic conductivity and its evolution along individual roots and with soil depth. We found that for both crops, root axial hydraulic conductivity, derived from root metaxylem structures, decreased with increasing distance from the root base. While the extent of the decrease was relatively similar in young roots of both crops, mature roots of alfalfa were 4-fold more conductive than intermediate wheatgrass roots in the field. In conclusion, alfalfa with greater root length at depth and greater root axial hydraulic conductivity was found more suited for deep water uptake in comparison to intermediate wheatgrass roots. Compared to other studies on intermediate wheatgrass, which have mainly focused on the first metre of soil (Sainju et al. 2017; de Oliveira et al. 2020), these results are relatively novel and give hope for the development of a perennial grain crop with enhanced use of deep stored water. In the case of alfalfa, the root growth and water uptake obtained in this study were much shallower than those found in the literature with reported changes in soil water content down to 10 m soil depth in some studies (Li and Huang 2008). At this study site, it is expected that crop growth was restricted by the high soil bulk density, elevated winter groundwater level and the relatively short growing season in comparison to other regions of the world. These results highlight the necessary research work of understanding crop physiological plasticity in relation to their growing environment. Interestingly, plastic response of the two crops were particularly identified between field and rhizobox conditions with significant differences in root anatomy observed. With this in mind, extrapolation of scientific results to agricultural practices can only be done with strong understanding of the influence of the research facility (Poorter et al. 2012) and of the GxExM interactions at play (Kirkegaard and Hunt 2010). Field research that are often associated to "real life" conditions do not escape from such rules.

Understanding hydraulic compensation at the plant level

The contribution of deep soil layers to crop transpiration was found to increase when the topsoil dries, during the spring-summer season for both crops. In the same way, model simulations predicted an increase in deep water uptake during the 2018 drought for both crops. These results suggest that a passive compensation is happening when topsoil dries but understanding the physiological processes behind such passive compensation is complex. Since the root system is a network, passive compensation of crop water uptake occurs when a certain layer of soil dries and therefore the plant water uptake is compensated/taken up in wetter soil layers (Thomas et al. 2020). Thus, at the plant level the extent of passive compensation depends on the root system hydraulic architecture. In particular deep water uptake and passive compensation depends on the amount of roots at depth (Kirkegaard et al. 2007) and on the root axial hydraulic conductivity (Garrigues et al. 2006; Pierret et al. 2006). Indeed, assuming that radial hydraulic conductivity of lateral roots in shallow and deep soil layers is similar, the factor limiting both water uptake and compensation of uptake at depth is the root axial hydraulic conductivity and the distribution of the hydraulic tension within the root system, for a given transpiration rate (Zwieniecki et al. 2003). Alfalfa with less root length in the topsoil, more root length in the subsoil and higher axial hydraulic conductivity seems more disposed for passive compensation to occur, in comparison to intermediate wheatgrass. As changes in root length density and axial hydraulic conductivity are minor within the first 50 cm of soil (State-of-the-Art: Figure 3 and Chapter 4), study on compensated water uptake should at least cover a minimum depth of 1.0 m to account for significant changes in root length density and root axial hydraulic conductivity.

While this study focused on belowground processes, aboveground processes should not be discarded. In particular, understanding the point at which deeper compensation of water uptake is no longer sufficient to support transpiration seems a key trait for drought resistance (Vandoorne et al. 2012). Once this point has been reached, active compensation, in the form of increased root growth at depth, could occur and reduce the effect of topsoil drying (Vandoorne et al. 2012).

However, this was not observed in our study. Understanding the processes behind both active and passive compensation is central to build more drought resilient and resistant cropping systems. These processes are very specific to the crop species and the environment and therefore require further research work.

Learnings on perennial crops

This study focused on perennial crops that are still understudied and poorly integrated into current agricultural systems. Alfalfa, due to its common use as a forage crop, soil nitrogen builder and deep water depletion, has received substantial attention from the scientific community (Dirksen and Raats 1985; Sheaffer et al. 1988; Dardanelli et al. 1997; Li and Huang 2008). In this study alfalfa did not grow as deeply and did not take up as much deep water as in other studies (Li and Huang 2008; Fan et al. 2016a) most likely due to soil conditions and limited total evaporative demand at this location. However, what is particularly interesting is to compare the results obtained for intermediate wheatgrass with those obtained for alfalfa. A major challenge for the development of perennial cropping systems is the development of profitable perennial grain crops. Intermediate wheatgrass is a promising substitute for wheat but the number of research studies is still limited. In fact, despite growing international attention on intermediate wheatgrass and its often mentioned deep root system (de Oliveira et al. 2020), this is one of the few studies, if not the first, that reports root growth, anatomy and water uptake of intermediate wheatgrass over such an extensive soil profile (>2.0 m). With evidence of deep root and water uptake during crucial physiological stages (i.e. after anthesis), permanent ground cover and potential for dual-purpose crop, producing forage as well as grains, intermediate wheatgrass is a very promising crop. In particular, its ability to use subsoil water late during the season and during drought events is very interesting for the development of drought resistant crops. From its deep root system, intermediate wheatgrass is also known for having enhanced nitrogen uptake capacity. A study by Sprunger et al. (2018) showed greater nitrogen use efficiency due to higher nitrogen utilisation and retention in intermediate wheatgrass than in annual winter wheat. However, it would be particularly interesting to compare the performance over years between intermediate wheatgrass and an annual crop/catch crop rotation as such systems also have high nitrogen use efficiency (Thorup-Kristensen 2006).

Contrary to dicotyledonous plants known to produce perennial main root structures, the longevity of roots of perennial grasses is less well known. The survival of the intermediate wheatgrass root system from one year to the next will be important for its effect on autumn and winter deep nitrogen uptake. In the short term, intermediate wheatgrass could be appealing to farmers who are seeking long-term ground cover on their fields. It could also be used as a dual-purpose crops and for rotational grazing practices (Pugliese 2017). With its dense root system in the topsoil and roots deep into the subsoil, it has characteristics that enable it to compete for resources in both the topsoil and the subsoil, which is interesting for intercropping practices. In this regard, testing the physiological response of intermediate wheatgrass when intercropped with shallow or deep-rooted crops, respectively, would be interesting to study the effect of root system complementarity (Fletcher et al. 2016). At the study site, extensive subsoil water extraction is an option because soil water fully recharge every autumn/winter. In areas with more limited subsoil water, growing deep rooted perennial crops, and particularly alfalfa, could induce a yield penalty to the subsequent crop. In some cases, complete recharge of the soil profile was estimated to take up to five years after the crop termination (McCallum et al. 2001). In the end, the long-term benefits of growing deep rooted perennial crops and extensive subsoil water extraction depends on the local conditions and water recharge and must be carefully considered (Passioura 1983).

Prospect for breeding a perennial grain crop

Because of its deep rooting system and water uptake, intermediate wheatgrass is a promising perennial grain crop with high potential for deep water usage. This study provides novel knowledge on the root system distribution and architecture, as well as on root anatomical characteristics and water use. This is particularly interesting for future breeding work and for the development of drought resistant cultivars (Lynch 2013, 2018; Dehaan et al. 2018). In particular, further breeding effort is required to increase grain yield as well as the amount of roots in the deepest soil layers. In doing so, the steep, cheap and deep ideotype seems particularly interesting to enhance subsoil exploration (Lynch 2013). Taking example from maize and barley, increasing the root growth at depth could originate from reducing the number of crown roots (Gao and Lynch 2016), reducing the number of cortical cell (Chimungu et al. 2014a) or having a metabolically cheaper root cortex due to the presence of aerenchyma (Chimungu et al. 2015) and/or root cortical senescence (Schneider et al. 2017). However, the relatively dimorphic root systems observed in this study could be interesting for both topsoil and subsoil foraging and is supposed to confer higher resilience in areas with combined drought and low phosphorus fertility (Burridge et al. 2020). Future breeding attempt could couple root system architecture to metaxylem vessels structure in order to develop cultivar with different water use strategy (i.e. conservative vs water spending) (Richards and Passioura 1989).

Relevance of the findings for research

This study illustrates that understanding crop differences in water uptake at great soil depth and under field conditions is challenging. From a research point of view, this study is interesting because it investigates the application of several known research methods in deep subsoil and field conditions. Despite their agricultural relevance, studies on root growth and root water uptake under field conditions and at great soil depths (i.e. below 1.0 m) are not frequent (Pierret et al. 2016). This is often due to the cost, labour and technology required to achieve the research work (Thorup-Kristensen et al. 2020a). This work, by combining several non-destructive and destructive methods, allows the evaluation of each approach. The main results are that (1) empirical methods on root water uptake have converged on the fact that alfalfa took up more water at depth than intermediate wheatgrass but differences in the amount of water extracted were observed between methods, (2) methods on root growth showed divergent information that requires particular understanding of the influence of the method on the growth conditions and the plant, (3) calibration of a model simulation using empirical measures seems the best approach to reach quantitative conclusions on root water uptake and (4) understanding the influence of sensor/research method on the plant functioning is particularly important to draw realistic conclusions.

TDR sensors have proven to be very useful for monitoring changes in soil moisture content over a long periods of time. However, it should be kept in mind that TDR sensor readings could be influenced by the soil conditions within their vicinity. Preferential root growth, preferential water flow and/or differences in soil texture or structure could occur at the soil-sensor interface and can have significant influence on the sensor readings. It is also important to note that they are an indirect measurement of plant and root water absorption. To obtain quantitative estimates of root water uptake from soil moisture measurements, a simulation model such as Hydrus-1D should be used to integrate the soil hydraulic properties and the various water fluxes that occur at the field and plant level. In this regard, it would have been particularly useful to place TDR sensors in shallow soil layers (e.g. 25 cm) for the calibration of Hydrus-1D.

The use of stable isotope techniques (i.e. natural abundance and labelled water) was found to be particularly useful for detecting uptake of water but requires understanding of the water movement and/or dilution of the tracer solution within the soil and the plant. Coupling of natural abundance and/or tracer studies to soil water movement models could allow more precise conclusions on root water uptake to be made (Sutanto et al. 2012; Mazzacavallo and Kulmatiski 2015; Zheng et al. 2018). However, in this study, this was not possible due to crop water samples (i.e. transpiration, leaf and stems) that were naturally too enriched. Indeed, enrichment of water at the plant level could, among other things originate from (1) backflow of enriched water in leaves (Dawson and Ehleringer 1993), (2) exchange of water from the phloem to the xylem (Farquhar et al. 2007), (3) mixing of water with different residence time and isotopic signature (Rothfuss and Javaux 2017a), (4) diurnal changes of plant water and air water vapor isotopic signature (Cernusak et al. 2016), (5) fractionation during root water uptake due to arbuscular mycorrhizas (Poca et al. 2019) and (6) fractionation during root water uptake due to differences in water binding to the soil (Vargas et al. 2017). Furthermore, the use of such methods requires two major assumptions to be made, that (1) water transport time in roots is negligible and (2) water redistribution within the soil is negligible during the time of experiment (Rothfuss and Javaux 2017b). For transpiration samples the volume and isotopic signature of the water vapor enclosed within the collection bag, placed around the plant, is likely to have a significant influence on the samples isotopic signature. However, this method proved to be straightforward and easy to use and is undoubtedly useful for labelling experiments. To date, sampling solely the xylem water is still challenging for herbaceous species and sampling the collar (e.g. tiller) or root at the base of the plant seems to be the best option for sampling the least contaminated water.

In the end, TDR sensor measurements and tracer labelling studies converged on the greater water uptake at depth for alfalfa in comparison to intermediate wheatgrass. However, the difference measured between the two species was very different depending on the method used. Indeed, differences between the two crops in terms of deep water uptake were greater when measured with isotopic tracers than with TDR sensors.

This study also shows that results obtained on root growth vary significantly depending on the method used. As discussed in chapter 4, both minirhizotrons and the soil coring method have pros and cons that are well discussed (e.g. presence of remnants roots, preferential root growth, compaction around the minirhizotron tube) (De Ruijter et al. 1996; Watt et al. 2008). The minirhizotron method seems more suited for studying root dynamics over time whereas the soil coring method seems more suited for quantification of root distribution along a soil profile (Johnson et al. 2001). However, the author wishes to emphasize the methodological advantage that the minirhizotron method still present. Nowadays, recent advances in artificial intelligence allow rapid processing of a large number of minirhizotron images, (approximately 35,000 images were processed in this study) (Smith et al. 2020). This opens up new perspectives for root research, specifically on root phenology (i.e. from their emergence to their death). This should be easily done in the future and would allow a better understanding of the root's capacity to use soil resources over time. Such knowledge is particularly necessary in the study of perennial grain crops where the continuity of the root system over time is still in question.

The use of simulation models made it possible to estimate quantitatively root water uptake. However, implementation of the model required some assumptions to be made that limit the interpretation of the results. The most important of these assumptions is the use of a fixed root distribution for both years. This constitutes an unrealistic hypothesis because root growth is a dynamic process and root length, particularly at depth, was found to be different between the two years in the case of alfalfa. In 2019 this constitutes a minor overestimation, as, at early stage of crop growth, water will preferentially be taken up from shallow soil layers.

Furthermore, the accuracy of the model depends on the ability to estimate the soil hydraulic characteristics. This is particularly challenging when considering deep soil layers. Accurately measuring soil hydraulic characteristics along a deep soil profile is particularly destructive and labour and time consuming. Inverse modelling allowed for the calibration of some of the soil hydraulic parameters. Nevertheless, optimizing more parameters would have led to unrealistic predictions, as the information from the two drying cycles is limited to only two soil depths. Increasing the amount of measured soil water content observations, especially in the topsoil, would most likely increase the model accuracy and erase small prediction errors. Greater characterization of soil hydraulic parameters would also allow for the optimization of others parameters, like root water uptake parameters (Hupet and Lambot 2003). The challenge is to accurately understand hydraulic conductance of the soil, the rhizosphere and of the different types of roots that vary with time and space (Baroni et al. 2010; Carminati et al. 2010; York et al. 2017). In attempting to do so, combination of results from root hydraulic architecture models with macroscopic models are needed to better understand the non-linear functions that governs water movement at the soil-root interface (Draye et al. 2010). However, very little is known on root hydraulic properties under field conditions, especially in deep soil layers. Three-dimensional models also provide new opportunities by considering more realistically the spatial heterogeneity of soil water availability for the plant, rather than an averaged value as in 1-dimensional models (de Willigen et al. 2012). Simulation of root water uptake at the plant and field level have made considerable progress in recent years and researchers are calling for a benchmark study (Schnepf et al. 2020), but higher consideration of subsoil and deep roots processes are highly needed.

Conclusion and perspectives

The aim of this thesis was to study deep root growth and water uptake of two perennial crops under field conditions. Both perennial crops were deep rooted with roots deeper than 2.0 m soil depth. Alfalfa presented greater root length at depth than intermediate wheatgrass. Interestingly, root length distribution differences between the two crops were higher when measured along minirhizotron tubes than with soil cores. Evolution of the soil water content and an isotope tracer labelling experiment showed that alfalfa took up more water below 1.5 m soil depth than intermediate wheatgrass. Even more, TDR sensors showed that alfalfa took up water down to 2.5 m soil depth in 2019. However, it is noteworthy to mention that roots of intermediate wheatgrass were still active in terms of water uptake at 2.0 m soil depth. Here again, the amount of deep water uptake was variable depending on the research method used.

Estimation of the contribution of deep soil layers to the plant transpiration was done using Hydrus-1D. Validation of the model was possible as simulated soil water movement and hydraulic characteristics matched measured values well. Although the use of a fixed root length distribution, measured in 2019 limits our conclusions on crop water uptake in 2018, the model provides a quantitative estimate of the differences in water uptake between the two crops and climatic conditions. Overall, alfalfa absorbed twice as much water below 1 m soil depth, than intermediate wheatgrass did. For both crops, the contribution of deep soil layers to the plant water uptake increased throughout both seasons reaching a peak around July. Furthermore, deep water uptake was predicted to increase under dry weather conditions like in 2018. These two results suggest that the presence of roots in deep soil layers allow passive compensation of water uptake from surface to deep soil layers to occur, thus mitigating the effect of topsoil drying. This is supported by the fact that isotopic tracer uptake were correlated to the root length density at depth in the case of alfalfa. In depth anatomical descriptions showed that axial hydraulic conductance was, on average, 4-fold greater in roots of field grown alfalfa than in roots of intermediate wheatgrass, due to the presence of bundles of secondary metaxylem vessels. For both crops and under field conditions, axial hydraulic conductance decreases with soil depth and roots below 1.75 m soil depth were, at least 3-fold and up to 600-fold, less conductive than topsoil roots. The magnitude of this decrease is expected to be a key factor in the plant's ability to absorb water from deep soil layers. In conclusion, higher stomatal conductance, greater root length at depth and higher axial hydraulic conductance are some of the physiological and anatomical characteristics identified in this study that explain the difference in deep water uptake between the two crops. Although alfalfa is taking more water from deep soil layers, performances of intermediate wheatgrass in subsoil exploration and deep water uptake showed interesting potential for future breeding of a drought resistant perennial crop.

This study, by combining many different growth conditions and research methods, illustrates the plasticity of plants in relation to their growing environment. In particular, root growth and anatomy were found to be relatively plastic traits depending on the growing environment (i.e. rhizoboxes vs field) or research method (i.e. minirhizotron vs soil coring). This constitutes an underlying challenge faced by all root researchers. Understanding the plant response to its growing environment and how research methods modify that environment, ultimately affecting the plant functioning, is particularly important in order to generate relevant conclusions. The best approach might be to combine results from multiple growing environments, multiple research methods or both when possible.

Deep rooting is a trait that is certainly common but highly variable because of complex genotypic, environmental and management synergies that apply. While understanding deep rooting and water uptake is certainly more challenging than one would think, understanding the role that perennial crops could play in current agricultural systems is complex in itself. Perennial cropping systems offer promising production and ecosystems benefits as well as an avenue for more efficient and resilient cropping systems. Their ability to make use of deep soil water, late during the season, seems particularly interesting. Further work is needed for exploitation of these characteristics by farmers. In particular, assessing the long-term effect of such extensive use of deep soil water requires particular attention. Long-term studies on perennial cropping system are highly needed.

Author's contribution

I am the first author of all the content found in this manuscript. Where quotations have been made, "quotation marks" have been used. During this PhD work I have received support from Hannah Schneider, Jonathan P. Lynch, Simon Fiil Svane, Dorte Bodin Dresbøll and Kristian Thorup-Kristensen for the conception and designed of my studies. I collected most of the data with support from Hannah Schneider on the root anatomy data and assistance from Joost Sleiderink on the isotope and soil coring campaign. I facilitated the collaboration between Joost Sleiderink and Efstathios Diamantopoulos for the implementation of the HYDRUS-1D model and finalised the simulation work with their support. Abraham George Smith implemented a convolutional neural network allowing the analysis of minirhizotron images and guided me in implementing the network. I analysed all the data and wrote this thesis. I received comments from all the co-authors on each of the manuscripts that will be published (i.e. Chapter III and IV). I received comments From John Kirkegaard and Kirsten Verburg on the second manuscript (i.e. Chapter IV) as part of the journal policy.

References

Ahmed MA, Kroener E, Benard P, et al (2016a) Drying of mucilage causes water repellency in the rhizosphere of maize: measurements and modelling. Plant Soil 407:161–171. doi: 10.1007/s11104-015-2749-1

Ahmed MA, Kroener E, Holz M, et al (2014) Mucilage exudation facilitates root water uptake in dry soils. Funct Plant Biol 41:1129–1137. doi: 10.1071/FP13330

Ahmed MA, Passioura J, Carminati A (2018a) Hydraulic processes in roots and the rhizosphere pertinent to increasing yield of water-limited grain crops: a critical review. J Exp Bot 69:3255–3265. doi: 10.1093/jxb/ery183

Ahmed MA, Zarebanadkouki M, Kaestner A, Carminati A (2016b) Measurements of water uptake of maize roots : the key function of lateral roots. Plant Soil 398:59–77. doi: 10.1007/s11104-015-2639-6

Ahmed MA, Zarebanadkouki M, Meunier F, et al (2018b) Root type matters: measurement of water uptake by seminal, crown, and lateral roots in maize. J Exp Bot 69:1199–1206. doi: 10.1093/jxb/erx439

Allen RG, Jensen ME, Wright JL, Burman RD (1989) Operational Estimates of Reference Evapotranspiration. Agron J 81:650–662. doi: 10.2134/agronj1989.00021962008100040019x

Allen RG, Pereira LS (1998) Crop evapotranspiration - Guidelines for computing crop water requirements - FAO Irrigation and drainage paper 56

Allen RG, Pruitt WO, Wright JL, et al (2006) A recommendation on standardized surface resistance for hourly calculation of reference ETo by the FAO56 Penman-Monteith method. Agric Water Manag 81:1–22. doi: 10.1016/j.agwat.2005.03.007

Amellal N, Burtin G, Bartoli F, Heulin T (1998) Colonization of Wheat Roots by an Exopolysaccharide-Producing Pantoea agglomerans Strain and Its Effect on Rhizosphere Soil Aggregation. Appl Environ Microbiol 64:3740–3747

Anderson-Taylor G, Marshall C (1983) Root-Tiller Interrelationships in Spring Barley (Hordeum distichum (L.) Lam.). Ann Bot 51:47–58

Angers DA, Caron J (1998) Plant-induced changes in soil structure: Processes and feeedbacks. Biogeochemistry 42:55–72

Angus JF, Gault RR, Peoples MB, et al (2001) Soil water extraction by dryland crops, annual pastures, and lucerne in south-eastern Australia. Aust J Agric Res 52:183–192

Aroca R, Amodeo G, Fernández-Illescas S, et al (2005) The Role of Aquaporins and Membrane Damage in Chilling and Hydrogen Peroxide Induced Changes in the Hydraulic Conductance of Maize Roots. Plant Physiol 137:341–353. doi: 10.1104/pp.104.051045.maize

Aroca R, Ruiz-lozano JM (2012) Chapter 4. Regulation of Root Water Uptake Under Drought Stress Conditions. In: Root water transport under abiotic stress conditions

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Myccorrhiza 11:3–42

Baligar VC, Fageria NK, Elrashidi MA (1998) Toxicity and Nutrient Constraints on Root Growth. HortScience 33:6

Baroni G, Facchi A, Gandolfi C, et al (2010) Uncertainty in the determination of soil hydraulic parameters and its influence on the performance of two hydrological models of different complexity. Hydrol Earth Syst Sci 14:251–270

Barraclough PB, Kuhlmann H, Weir AH (1989) The Effects of Prolonged Drought and Nitrogen Fertilizer on Root and Shoot Growth and Water Uptake by Winter Wheat. J Agron Crop Sci 360:352–361

Barraclough PB, Leigh RA (1984) The growth and activity of winter wheat roots in the field: the effect of sowing date and soil type on root growth of high-yielding crops. J Agric Sci 103:59–74

Baskerville GL, Emin P (1969) Rapid Estimation of Heat Accumulation from Maximum and

Minimum Temperatures. Ecology 50:514–517

Bates TR, Lynch JP (2001) Root hairs confer a competitive advantage under low phosphorus availability. Plant Soil 236:243–250

Bauerle TL, Richards JH, Smart DR, Eissenstat DM (2008) Importance of internal hydraulic redistribution for prolonging the lifespan of roots in dry soil. Plant Cell Environ 31:177–186. doi: 10.1111/j.1365-3040.2007.01749.x

Baxter I, Hosmani PS, Rus A, et al (2009) Root Suberin Forms an Extracellular Barrier That Affects Water Relations and Mineral Nutrition in Arabidopsis. PLoS Genet 5:1–12. doi: 10.1371/journal.pgen.1000492

Bazzaz FA, Chiariello NR, Coley PD, Pitelka LF (1987) Allocating Resources to Reproduction and Defense in plants are relating ecological roles to resource use. Biogeosciences 37:58–67

Becker R, Wagoner P, Hanners GD, Saunders RM (1991) Compositional, nutritional and functional evaluation of intermediate wheatgrass (Thinopyrum intermedium). J Food Process Preserv 15:63–77

Bengough AG (2012) Root elongation is restricted by axial but not by radial pressures: So what happens in field soil? Plant Soil 360:15–18. doi: 10.1007/s11104-012-1428-8

Bergmann J (2017) Root traits and their effect in plant-soil interactions

Bishopp A, Lynch JP (2015) The hidden half of crop yields. Nat Plants 1:1–2. doi: 10.1038/nplants.2015.117

Blair G (1993) Nutrient efficiency - what do we really mean? In: Genetic aspects of plant mineral nutrition. pp 205–213

Bloom AJ, Chapin SF, Mooney HA (1985) Resource limitation in plants - An economic analogy. Annu Rev Ecol Syst 16:363–92

Blum A (2009) Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. F Crop Res 112:119–123. doi: 10.1016/j.fcr.2009.03.009

Blum A, Ramaiah S, Kanemasu ET, Paulsen GM (1990) Wheat recovery from drought stress at the tillering stage of development. F Crop Res 24:67–85

Borg H, Grimes DW (1986) Depth Development of Roots with Time: An Empirical Description. Am Soc Agric Eng 29:194–197. doi: 10.13031/2013.30125

Botwright Acuña TL, Pasuquin E, Wade LJ (2007) Genotypic differences in root penetration ability of wheat through thin wax layers in contrasting water regimes and in the field. Plant Soil 301:135–149. doi: 10.1007/s11104-007-9428-9

Bramley H, Turner NC, Turner DW, Tyerman SD (2009) Roles of Morphology, Anatomy, and Aquaporins in Determining Contrasting Hydraulic Behavior of Roots. Plant Physiol 150:348–364. doi: 10.1104/pp.108.134098

Bremner PM, Fazekas de St. Groth C (1986) A Field Comparison of Sunflower (Helicanthus annuus) and Sorghum (Sorghum bicolor) in a Long Drying Cycle. I. Water Extraction. Aust J Agric Res 37:483–493

Brodersen CR, McElrone AJ (2013) Maintenance of xylem network transport capacity: A review of embolism repair in vascular plants. Front Plant Sci 4:1–11. doi: 10.3389/fpls.2013.00108

Burridge JD, Findeis JL, Jochua CN, et al (2019) A case study on the efficacy of root phenotypic selection for edaphic stress tolerance in low-input agriculture: Common bean breeding in Mozambique. F Crop Res 244:107612. doi: 10.1016/j.fcr.2019.107612

Burridge JD, Rangarajan H, Lynch JP (2020) Comparative phenomics of annual grain legume root architecture. Crop Sci 60:2574–2593. doi: 10.1002/csc2.20241

Cai G, Vanderborght J, Couvreur V, et al (2017) Parameterization of Root Water Uptake Models Considering Dynamic Root Distributions and Water Uptake Compensation. Vadoze Zo J 17:160125. doi: 10.2136/vzj2016.12.0125

Caldwell MM, Richards JH (1998) Hydraulic Lift: Consequences of Water Efflux from the Roots of Plants. Oecologia 113:151–161. doi: 10.1007/s004420050363

Canadell J, Jackson R, Ehleringer J, et al (1996) Maximum rooting depth of vegetation types at the global scale. Oecologia 108:583–595. doi: 10.1007/BF00329030

Caradus JR, Evans PS (1977) Seasonal root formation of white clover, ryegrass, and cocksfoot in New Zealand. New Zeal J Agric Res 20:337–342. doi: 10.1080/00288233.1977.10427344

Carminati A (2013) Rhizosphere wettability decreases with root age: a problem or a strategy to increase water uptake of young roots ? Front Plant Sci 4:1–9. doi: 10.3389/fpls.2013.00298

Carminati A, Benard P, Ahmed MA (2017a) Liquid bridges at the root-soil interface. Plant Soil 417:1–15. doi: 10.1007/s11104-017-3227-8

Carminati A, Moradi AB, Vetterlein D, et al (2010) Dynamics of soil water content in the rhizosphere. Plant Soil 332:163–176. doi: 10.1007/s11104-010-0283-8

Carminati A, Passioura JB, Zarebanadkouki M, et al (2017b) Root hairs enable high transpiration rates in drying soils. New Phytol 216:771–781. doi: 10.1111/nph.14715

Carminati A, Vetterlein D (2013) Plasticity of rhizosphere hydraulic properties as a key for efficient utilization of scarce resources. Ann Bot 112:277–290. doi: 10.1093/aob/mcs262

Carminati A, Vetterlein D, Koebernick N, et al (2013) Do roots mind the gap? Plant Soil 367:651–661. doi: 10.1007/s11104-012-1496-9

Carvajal M, Cooke DT, Clarkson DT (1996) Responses of wheat plants to nutrient deprivation may involve the regulation of water-channel function. Planta 199:372–381

Cernusak LA, Barbour MM, Arndt SK, et al (2016) Stable isotopes in leaf water of terrestrial plants. Plant Cell Environ 39:1087–1102. doi: 10.1111/pce.12703

Chaumont F, Tyerman SD (2014) Aquaporins: Highly Regulated Channels Controlling Plant Water Relations. Plant Physiol 164:1600–1618. doi: 10.1104/pp.113.233791

Chen HYH, Brassard BW (2013) Intrinsic and Extrinsic Controls of Fine Root Life Span. CRC Crit Rev Plant Sci 32:151–161. doi: 10.1080/07352689.2012.734742

Chen T, Cai X, Wu X, et al (2011) Casparian strip development and its potential function in salt tolerance. Plant Signal Behav 6:1499–1502. doi: 10.4161/psb.6.10.17054

Chimungu JG, Brown KM, Lynch JP (2014a) Reduced Root Cortical Cell File Number Improves Drought Tolerance in Maize. Plant Physiol 166:1943–1955. doi: 10.1104/pp.114.249037

Chimungu JG, Brown KM, Lynch JP (2014b) Large Root Cortical Cell Size Improves Drought. Plant Physiol 166:2166–2178. doi: 10.1104/pp.114.250449

Chimungu JG, Maliro MFA, Nalivata PC, et al (2015) Utility of root cortical aerenchyma under water limited conditions in tropical maize (Zea mays L.). F Crop Res 171:86–98. doi: 10.1016/j.fcr.2014.10.009

Choi W, Roberts DM (2007) Arabidopsis NIP2;1, a Major Intrinsic Protein Transporter of Lactic Acid Induced by Anoxic Stress. J Biol Chem 282:24209–24218. doi: 10.1074/jbc.M700982200

Christmann A, Weiler EW, Steudle E, et al (2007) A hydraulic signal in root-to-shoot signalling of water shortage. Plant J 52:167–174. doi: 10.1111/j.1365-313X.2007.03234.x

Clark ID, Fritz P (1997) Environmental Isotopes in Hydrogeology

Clément C, Schneider Hannah M DDB, P LJ, Kristian T-K Root and xylem anatomy varies with root length, root order, soil depth, and environment in intermediate wheatgrass Thinopyrum intermedium and Alfalfa Medicago sativa. F Crop Res

Colmer TD (2003) Aerenchyma and an Inducible Barrier to Radial Oxygen Loss Facilitate Root Aeration in Upland, Paddy and Deep-water Rice (Oryza sativa L.). Ann Bot 91:301–309. doi: 10.1093/aob/mcf114

Colombi T, Braun S, Keller T, Walter A (2017) Artificial macropores attract crop roots and enhance plant productivity on compacted soils. Sci Total Environ 574:1283–1293. doi: 10.1016/j.scitotenv.2016.07.194

Comstock JP (2002) Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. J Exp Bot 53:195–200

Couvreur V, Faget M, Lobet G, et al (2018) Going with the Flow: Multiscale Insights into the Composite Nature of Water Transport in Roots. Plant Physiol 178:1689–1703. doi: 10.1104/pp.18.01006

Cox TS, Glover JD, Van Tassel DL, et al (2006) Prospects for Developing Perennial Grain Crops. Bios 56:649–659

Crews TE (2005) Perennial crops and endogenous nutrient supplies. Renew Agric Food Syst 20:25–37. doi: 10.1079/RAF200497

Crews TE, Blesh J, Culman SW, et al (2016) Going where no grains have gone before: From early to mid-succession. Agric Ecosyst Environ 223:223–238. doi: 10.1016/j.agee.2016.03.012

Crews TE, Dehaan LR (2015) The Strong Perennial Vision: A Response. Agroecol Sustain Food Syst 39:500–515. doi: 10.1080/21683565.2015.1008777

Crews TE, Peoples MB (2005) Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems ? A review. Nutr Cycl Agroecosystems 72:101–120. doi: 10.1007/s10705-004-6480-1

Culman SW, Dupont ST, Glover JD, et al (2010) Long-term impacts of high-input annual cropping and unfertilized perennial grass production on soil properties and belowground food webs in Kansas, USA. Agric Ecosyst Environ 137:13–24. doi: 10.1016/j.agee.2009.11.008

Culman SW, Snapp S, Ollenburger M, Basso B (2013) Soil and Water Quality Rapidly Responds to the Perennial Grain Kernza Wheatgrass. Agron J 105:735–744. doi: 10.2134/agronj2012.0273

Cutforth HW, Angadi S V, McConkey BG, et al (2009) Comparing plant water relations for wheat with alternative pulse and oilseed crops grown in the semiarid Canadian prairie. Can J Plant Sci 89:823–835. doi: 10.4141/CJPS08138

da Silva AP, Kay BD (1997) Effect of soil water content variation on the least limiting water range. Soil Sci Soc Am J 61:884–888. doi: 10.2136/sssaj1997.03615995006100030024x

Dardanelli JL, Bachmeier OA, Sereno R, Gil R (1997) Rooting depth and soil water extraction patterns of different crops in a silty loam haplustoll. F Crop Res 54:29–38. doi: 10.1016/S0378-4290(97)00017-8

Davis MR (1991) The comparative phosphorus requirements of some temperate perennial legumes. Plant Soil 133:17–30

Dawson TE, Ehleringer JR (1993) Isotopic enrichment of water in the “ woody ” tissues of plants: Implications for plant water source, water uptake, and other studies which use the stable isotopic composition of cellulose. Geochim Cosmochim Acta 57:3487–3492

de Oliveira G, Brunsell NA, Crews TE, et al (2020) Carbon and water relations in perennial Kernza (Thinopyrum intermedium): An overview. Plant Sci 295:110279. doi: 10.1016/j.plantsci.2019.110279

De Ruijter FJ, Veen BW, Van Oijen M (1996) A comparison of soil core sampling and minirhizotrons to quantify root development of field-grown potatoes. Plant Soil 182:301–312

de Willigen P, van Dam JC, Javaux M, Heinen M (2012) Root water uptake as simulated by three soil water flow models. Vadose Zo J 11:1–31

Dehaan L, Christians M, Crain J, Poland J (2018) Development and Evolution of an Intermediate Wheatgrass Domestication Program. Sustainability 10:1–19. doi: 10.3390/su10051499

Den Herder G, Van Isterdael G, Beeckman T, De Smet I (2010) The roots of a new green revolution. Trends Plant Sci 15:600–607. doi: 10.1016/j.tplants.2010.08.009

Dexter AR (2004) Soil physical quality Part I . Theory, effects of soil texture, density, and organic matter, and effects on root growth. Geoderma 120:201–214. doi: 10.1016/j.geodermaa.2003.09.005

Dezsi Ş, Mîndrescu, Petrea D, et al (2018) High-resolution projections of evapotranspiration and water availability for Europe under climate change. Int J Climatol 38:3832–3841. doi: 10.1002/joc.5537

Diamantopoulos E, Iden SC, Durner W (2012) Inverse modeling of dynamic nonequilibrium in water flow with an effective approach. Water Resour Res 48:1–16. doi: 10.1029/2011WR010717

Dietrich D, Pang L, Kobayashi A, et al (2017) Root hydrotropism is controlled via a cortex-specific growth mechanism. Nat Plants 3:17057. doi: 10.1038/nplants.2017.57

Dirksen C, Raats PAC (1985) Water Uptake and Release by Alfalfa Roots. Agron J 77:. doi: 10.2134/agronj1985.00021962007700040025x

Draye X, Kim Y, Lobet G, Javaux M (2010) Model-assisted integration of physiological and environmental constraints affecting the dynamic and spatial patterns of root water uptake from soils. J Exp Bot 61:2145–2155. doi: 10.1093/jxb/erq077

Drew MC, Saker LR (1978) Nutrient Supply and the Growth of the Seminal Root System in Barley. III. Compensation increases in growth of lateral roots and in rates of phosphate uptake in response to a localized supply of phosphate. J Exp Bot 29:435–451

Drew MC, Saker LR (1975) Nutrient Supply and the Growth of the Seminal Root System in Barley. II. Localized compensatory increase in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of the root system. J Exp Bot 26:79–90

Dupont ST, Beniston J, Glover JD, et al (2014) Root traits and soil properties in harvested perennial grassland, annual wheat, and never-tilled annual wheat. Plant Soil 381:405–420. doi: 10.1007/s11104-014-2145-2

Durner W (1994) Hydraulic conductivity estimation for soils with heterogeneous pore structure. Water Resour Res 30:211–223

Ehleringer JR, Osmond CB (1989) Stable isotopes. In: Plant Physiological ecology. pp 281–300

Ehlert C, Maurel C, Tardieu F, Simonneau T (2009) Aquaporin-Mediated Reduction in Maize Root Hydraulic Conductivity Impacts Cell Turgor and Leaf Elongation Even without Changing Transpiration. Plant Physiol 150:1093–1104. doi: 10.1104/pp.108.131458

Eissenstat DM, Yanai RD (1997) The Ecology of Root Lifespan. Adv Ecol Res 27:1–60

Enstone DE, Peterson CA, Ma F (2003) Root Endodermis and Exodermis: Structure, Function, and Responses to the Environment. J Plant Growth Regul 21:335–351. doi: 10.1007/s00344-003-0002-2

Erice G, Louahlia S, Irigoyen JJ, et al (2010) Biomass partitioning, morphology and water status of four alfalfa genotypes submitted to progressive drought and subsequent recovery. J Plant Physiol 167:114–120. doi: 10.1016/j.jplph.2009.07.016

Fageria NK, Moreira A (2011) The Role of Mineral Nutrition on Root Growth of Crop Plants, 1st edn. Elsevier Inc.

Fan J, Du Y, Wang B, et al (2016a) Forage yield, soil water depletion, shoot nitrogen and phosphorus uptake and concentration, of young and old stands of alfalfa in response to nitrogen and phosphorus fertilisation in a semiarid environment. F Crop Res 198:247–257. doi: 10.1016/j.fcr.2016.08.014

Fan J, McConkey B, Wang H, Janzen H (2016b) Root distribution by depth for temperate agricultural crops. F Crop Res 189:68–74. doi: 10.1016/j.fcr.2016.02.013

Fan M, Bai R, Zhao X, Zhang J (2007) Aerenchyma Formed Under Phosphorus Deficiency Contributes to the Reduced Root Hydraulic Conductivity in Maize Roots. J Integr Plant Biol 49:598–604. doi: 10.1111/j.1672-9072.2007.00450.x

FAO (2013) Perennial Crops for Food Security Proceedings of the FAO Expert Workshop

FAO (2006) Guidelines for soil description

Farquhar GD, Cernusak LA, Barnes B (2007) Heavy Water Fractionation during Transpiration. Plant Physiol 143:11–18. doi: 10.1104/pp.106.093278

Feddes RA, Raats PAC (2004) Parameterizing the soil – water – plant root system. In: Feddes, R.A., de Rooij, G.H., van Dam, J.C. (Eds.), Proceedings of the Unsaturated Zone Modelling: Progress, Challenges and Applications.

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary Production of the Biosphere : Integrating Terrestrial and Oceanic Components. Science (80-) 281:237–240

Fischer RA, Connor DJ (2018) Issues for cropping and agricultural science in the next 20 years. F Crop Res 222:121–142. doi: 10.1016/j.fcr.2018.03.008

Fletcher AL, Kirkegaard JA, Peoples MB, et al (2016) Prospects to utilise intercrops and crop variety mixtures in mechanised, rain-fed, temperate cropping systems. Crop Pasture Sci 67:1252–1267

Fort F, Volaire F, Guilioni L, et al (2017) Root traits are related to plant water-use among rangeland Mediterranean species. Funct Ecol 31:1700–1709. doi: 10.1111/1365-2435.12888

Frank DA, Kuns MM, Guido DR (2002) Consumer control of grassland plant production. Ecology 83:602–606

Fransen B, Kroon HDE (2001) Long-term disadvantages of selective root placement: root proliferation and shoot biomass of two perennial grass species in a 2-year experiment. J Ecol 89:711–722

French RJ, Schultz JE (1984) Water Use Efficiency of Wheat in a Mediterranean-type Environment. I. The Relation between Yield, Water Use and Climate. Aust J Agric Res 35:743–64. doi: 10.1071/AR9840765

Frensch J, Steudle E (1989) Axial and Radial Hydraulic Resistance to Roots of Maize (Zea mays L.). Plant Physiol 91:719–726. doi: 10.1104/pp.91.2.719

Fukai S, Cooper M (1995) Development of drought-resistant cultivars using physio-morphological traits in rice. F Crop Res 40:67–86

Fulton LM, Lynd LR, Körner A, et al (2015) The need for biofuels as part of a low carbon energy future. Biofuels, Bioprod Biorefining 9:476–483. doi: 10.1002/bbb

Galindo-Castañeda T, Brown KM, Lynch JP (2018) Reduced root cortical burden improves growth and grain yield under low phosphorus availability in maize. Plant Cell Environ 41:1579–1592. doi: 10.1111/pce.13197

Gambetta GA, Fei J, Rost TL, et al (2013) Water Uptake along the Length of Grapevine Fine Roots: Developmental Anatomy, Tissue-Specific Aquaporin Expression, and Pathways of Water Transport. Plant Physiol 163:1254–1265. doi: 10.1104/pp.113.221283

Gao W, Hodgkinson L, Jin K, et al (2016) Deep roots and soil structure. Plant Cell Environ 39:1662–1668. doi: 10.1111/pce.12684

Gao Y, Lynch JP (2016) Reduced crown root number improves water acquisition under water deficit stress in maize (Zea mays L.). J Exp Bot 67:4545–4557. doi: 10.1093/jxb/erw243

Garnett T, Conn V, Kaiser BN (2009) Root based approaches to improving nitrogen use. Plant Cell Physiol 32:1272–1283. doi: 10.1111/j.1365-3040.2009.02011.x

Garrigues E, Doussan C, Pierret A (2006) Water Uptake by Plant Roots: I. Formation and Propagation of a Water Extraction Front in Mature Root Systems as Evidenced by 2D Light Transmission Imaging. Plant Soil 283:83–98. doi: 10.1007/s11104-004-7903-0

Gaudin ACM, Tolhurst TN, Ker AP, et al (2015) Increasing crop diversity mitigates weather variations and improves yield stability. PLoS One 10:1–20. doi: 10.1371/journal.pone.0113261

Gazis C, Feng X (2004) A stable isotope study of soil water: evidence for mixing and preferential flow paths. Geoderma 119:97–111. doi: 10.1016/S0016-7061(03)00243-X

Ge Z, Rubio G, Lynch JP (2000) The importance of root gravitropism for inter-root competition and phosphorus acquisition efficiency: results from a geometric simulation model. Plant Soil 218:159–171

Geldner N (2013) The Endodermis. Annu Rev Plant Biol 64:531–558. doi: 10.1146/annurev-arplant-050312-120050

George E, Horst WJ, Neumann E (2012) Adaptation of Plants to Adverse Chemical Soil Conditions. Elsevier Ltd

Glover JD, Culman SW, Dupont ST, et al (2010a) Harvested perennial grasslands provide ecological benchmarks for agricultural sustainability. Agric Ecosyst Environ 137:3–12. doi: 10.1016/j.agee.2009.11.001

Glover JD, Reganold JP, Bell LW, et al (2010b) Increased Food and Ecosystem. Sci Policy Forum 328:1638–1640

Goins GD, Russelle MP (1996) Fine root demography in alfalfa (Medicago sativa L.). Plant Soil 185:281–291

González-Paleo L, Vilela AE, Ravetta DA (2016) Back to perennials: Does selection enhance tradeoffs between yield and longevity? Ind Crops Prod 91:272–278. doi: 10.1016/j.indcrop.2016.07.018

Gourley CJP, Allan DL, Russelle MP (1994) Plant nutrient efficiency: A comparison of definitions and suggested improvement. Plant Soil 158:29–37

Grandy AS, Robertson GP (2007) Land-Use Intensity Effects on Soil Organic Carbon Accumulation Rates and Mechanisms. Ecosystems 2:58–73. doi: 10.1007/s10021-006-9010-

y

Gregory PJ (2006) Roots, rhizosphere and soil: the route to a better understanding of soil science ? Eur J Soil Sci 57:2–12. doi: 10.1111/j.1365-2389.2005.00778.x

Gregory PJ, McGowan M, Biscoe PV, Hunter B (1979) Water relations of winter wheat. J Agric Sci 93:494. doi: 10.1017/S0021859600038223

Grierson C, Nielsen E, Ketelaar T, Schiefelbein J (2014) Root Hairs. Am Soc Plant Biol 1–25. doi: 10.1199/tab.0172

Grondin A, Mauleon R, Vadez V, Henry A (2016) Root aquaporins contribute to whole plant water fluxes under drought stress in rice (Oryza sativa L .). Plant Cell Environ 39:347–365. doi: 10.1111/pce.12616

Hachez C, Veselov D, Ye Q, et al (2012) Short-term control of maize cell and root water permeability through plasma membrane aquaporin isoforms. Plant Cell Environ 35:185–198. doi: 10.1111/j.1365-3040.2011.02429.x

Haling RE, Brown LK, Bengough AG, et al (2014) Root hair length and rhizosheath mass depend on soil porosity, strength and water content in barley genotypes. Planta 239:643–651. doi: 10.1007/s00425-013-2002-1

Hall B, Lanba A, Lynch JP (2019) Three-dimensional analysis of biological systems via a novel laser ablation technique. J Laser Appl 31:1–5. doi: 10.2351/1.5096089

Hamblin A, Tennant D (1987) Root Length Density and Water-Uptake in Cereals and Grain Legumes - How Well Are They Correlated. Aust J Agric Res 38:513–527

Hamidov A, Helming K, Bellocchi G, et al (2018) Impacts of climate change adaptation options on soil functions: A review of European case-studies. L Degrad Dev 29:2378–2389. doi: 10.1002/ldr.3006

Hammac WA, Pan WL, Bolton RP, Koenig RT (2011) High resolution imaging to assess oilseed species' root hair responses to soil water stress. Plant Soil 339:125–135. doi: 10.1007/s11104-010-0335-0

Han E, Kautz T, Pude R (2015) Quantification of soil biopore density after perennial fodder cropping. Plant Soil 394:73–85. doi: 10.1007/s11104-015-2488-3

Henry A, Cal AJ, Batoto TC, et al (2012) Root attributes affecting water uptake of rice (Oryza sativa). J Exp Bot 63:695–709. doi: 10.1093/jxb/err313

Herzog M, Striker GG, Colmer TD, Pedersen O (2016) Mechanisms of waterlogging tolerance in wheat - a review of root and shoot physiology. Plant Cell Environ 39:1068–1086. doi: 10.1111/pce.12676

Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321:117–152. doi: 10.1007/s11104-008-9885-9

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Calif Agr Expt Sta Circ 347:1–32. doi: citeulike-article-id:9455435

Hodge A (2006) Plastic plants and patchy soils. J Exp Bot 57:401–411. doi: 10.1093/jxb/eri280

Hodge A (2004) The plastic plant: Root responses to heterogeneous supplies of nutrients. New Phytol 162:9–24. doi: 10.1111/j.1469-8137.2004.01015.x

Hodge A, Berta G, Doussan C, et al (2009) Plant root growth, architecture and function. Plant Soil 321:153–187. doi: 10.1007/s11104-009-9929-9

Holbrook NM, Zwieniecki MA (2005) Integration of Long Distance Transport Systems in Plants: Perspectives and Prospects for Future Research. Vasc Transp Plants 26:537–545. doi: 10.1016/B978-0-12-088457-5.50028-9

Hopmans JW, Šimůnek J (1999) Review of inverse estimation of soil hydraulic properties. In: Proceedings of the International Workshop on Characterization and Measurements of the Hydraulic Properties of Unsaturated Porous Media, edited by M. T. van Genuchten, F. J. Leij, and L. Wu, pp. 643–660, Univ. of Calif., Riverside, 1999. pp 643–659

Hose E, Clarkson DT, Steudle E, et al (2001) The exodermis: a variable apoplastic barrier. J Exp Bot 52:2245–2264

Hu B, Henry A, Brown KM, Lynch JP (2014) Root cortical aerenchyma inhibits radial nutrient transport in maize (Zea mays). Ann Bot 113:181–189. doi: 10.1093/aob/mct259

Hund A, Richner W, Soldati A, et al (2007) Root morphology and photosynthetic performance of maize inbred lines at low temperature. Eur J Agron 27:52–61. doi: 10.1016/j.eja.2007.01.003

Hund A, Ruta N, Liedgens M (2009) Rooting depth and water use efficiency of tropical maize inbred lines, differing in drought tolerance. Plant Soil 318:311–325. doi: 10.1007/s11104-008-9843-6

Hunt JR, Kirkegaard JA (2011) Re-evaluating the contribution of summer fallow rain to wheat yield in southern Australia. Crop Pasture Sci 62:915–929. doi: 10.1071/CP11268

Hunt JR, Lilley JM, Trevaskis B, et al (2019) Early sowing systems can boost Australian wheat yields despite recent climate change. Nat Clim Chang 9:244–247. doi: 10.1038/s41558-019-0417-9

Hunter MC, Smith RG, Schipanski ME, et al (2017) Agriculture in 2050 : Recalibrating Targets for Sustainable Intensification. Bioscience 67:386–391. doi: 10.1093/biosci/bix010

Hupet F, Lambot S (2003) Estimation of root water uptake parameters by inverse modeling with soil water content data. Water Resour Res 39:1–16. doi: 10.1029/2003WR002046

Jaramillo E, Nord EA, Chimungu JG, et al (2013) Root cortical burden influences drought tolerance in maize. Ann Bot 112:429–437. doi: 10.1093/aob/mct069

Jarvis NJ (1989) A simple empirical model of root water uptake. J Hydrol 107:57–72

Javelle M, Vernoud V, Rogowsky PM, Ingram GC (2011) Tansley review. Epidermis: the formation and functions of a fundamental plant tissue. New Phytol 189:17–39

Javot H, Maurel C (2002) The Role of Aquaporins in Root Water Uptake. Ann Bot 90:301–313. doi: 10.1093/aob/mcf199

Jenkinson DS, Potts JM, Perry JN, et al (1994) Trends in herbage yields over the last century on the Rothamsted Long-term Continuous Hay Experiment. J Agric Sci 122:365–374

Jenkinson DS, Poulton PR, Johnston AE, Powlson DS (2004) Turnover of Nitrogen-15-Labeled Fertilizer in Old Grassland. Soil Sci Soc Am J 875:865–875

Jin K, Shen J, Ashton RW, et al (2013) How do roots elongate in a structured soil? J Exp Bot 64:4761–4777. doi: 10.1093/jxb/ert286

Johnson DM, Sherrard ME, Domec J-C, Jackson RB (2014) Role of aquaporin activity in regulating deep and shallow root hydraulic conductance during extreme drought. Trees 28:1323–1331. doi: 10.1007/s00468-014-1036-8

Johnson MG, Tingey DT, Phillips DL, Storm MJ (2001) Advancing fine root research with minirhizotrons. Environ Exp Bot 45:263–289

Jones H, Leigh RA, Wyn Jones RG, Tomos AD (1988) The integration of whole-root and cellular hydraulic conductivities in cereal roots. Planta 174:1–7. doi: 10.1007/BF00394866

Jungers JM, Dehaan LH, Mulla DJ, et al (2019) Reduced nitrate leaching in a perennial grain crop compared to maize in the Upper Midwest, USA. Agric Ecosyst Environ 272:63–73. doi: 10.1016/j.agee.2018.11.007

Juniper BE, Barlow PW (1969) The Distribution of Plasmodesmata in the Root Tip of Maize. Planta 89:352–360

Kadam NN, Yin X, Bindraban PS, et al (2015) Does Morphological and Anatomical Plasticity during the Vegetative Stage Make Wheat More Tolerant of Water Deficit Stress Than Rice ? Plant Physiol Ecol 167:1389–1401. doi: 10.1104/pp.114.253328

Kantar MB, Tyl CE, Dorn KM, et al (2016) Perennial Grain and Oilseed Crops. Ann Rev plant Biol 67:703–29. doi: 10.1146/annurev-arplant-043015-112311

Kaspar TC, Bland WL (1992) Soil temperature and root growth. Soil Sci 154:290–299

Kattge J (2020) TRY plant trait database – enhanced coverage and open access. Glob Chang Biol 26:119–188. doi: 10.1111/gcb.14904

Kautz T (2014) Research on subsoil biopores and their functions in organically managed soils: A review. Renew Agric Food Syst 30:318–327. doi: 10.1017/S1742170513000549

Kirkegaard JA, Hunt JR (2010) Increasing productivity by matching farming system management and genotype in water-limited environments. J Exp Bot 61:4129–4143. doi: 10.1093/jxb/erq245

Kirkegaard JA, Hunt JR, McBeath TM, et al (2014) Improving water productivity in the Australian Grains industry — a nationally coordinated approach. Crop Pasture Sci 65:583–601

Kirkegaard JA, Lilley JM, Howe GN, Graham JM (2007) Impact of subsoil water use on wheat yield. Aust J Agric Res 58:303–315. doi: 10.1071/AR06285

Kirkegaard JA, Lilley JM, Hunt JR, et al (2015) Effect of defoliation by grazing or shoot removal on the root growth of field-grown wheat (Triticum aestivum L.). Crop Pasture Sci 66:249–259. doi: 10.1071/CP14241

Kirkham MB (2014) Chapter 15: Root Anatomy and Poiseuille's Law for Water Flow in Roots. In: Principles of Soil and Plant Water relations (2nd edition). pp 243–266

Knipfer T, Fricke W (2011) Water uptake by seminal and adventitious roots in relation to whole-plant water flow in barley (Hordeum vulgare L.). J Exp Bot 62:717–733. doi: 10.1093/jxb/erq312

Kobata T (1996) Contributions of capacity for soil water extraction and water use efficiency to maintenance of dry matter production in rice subjected to Drought. Japanese J Crop Sci 65:652–662. doi: 10.1626/jcs.65.652

Kochian L V, Piñeros MA, Liu J, Magalhaes J V (2015) Plant Adaptation to Acid Soils: The Molecular Basis for Crop Aluminum Resistance. Ann Rev plant Biol 66:571–98. doi: 10.1146/annurev-arplant-043014-114822

Koester RP, Skoneczka JA, Cary TR, et al (2014) Historical gains in soybean (Glycine max Merr.) seed yield are driven by linear increases in light interception, energy conversion, and partitioning efficiencies. J Exp Bot 65:3311–3321. doi: 10.1093/jxb/eru187

Koevoets IT, Venema JH, Elzenga JTM, Testerink C (2016) Roots Withstanding their Environment: Exploiting Root System Architecture Responses to Abiotic Stress to Improve Crop Tolerance. Front Plant Sci 7:1–19. doi: 10.3389/fpls.2016.01335

Kondo M, Murty MVR, Aragones D V. (2000) Characteristics of root growth and water uptake from soil in upland rice and maize under water stress. Soil Sci Plant Nutr 46:721–732. doi: 10.1080/00380768.2000.10409137

Korte CJ, Chu AC. (1983) Some effects of drought on perennial ryegrass swards. Proc new Zeal Grassl Assoc 44:211–216

Kramer PJ (1983) Water relations of plants

Kristensen HL, Thorup-Kristensen K (2004) Root Growth and Nitrate Uptake of Three Different Catch Crops in Deep Soil Layers. Soil Sci Soc Am J 68:529–537. doi: 10.2136/sssaj2004.5290

Kristensen HL, Thorup-Kristensen K (2007) Effects of vertical distribution of soil inorganic nitrogen on root growth and subsequent nitrogen uptake by field vegetable crops. Soil Use Manag 23:338–347. doi: 10.1111/j.1475-2743.2007.00105.x

Kumar R, Shankar V, Jat MK (2014) Evaluation of root water uptake models – a review. ISH J Hydraul Eng 21:115–124. doi: 10.1080/09715010.2014.981955

Kummu M, Guillaume JHA, de Moel H, et al (2016) The world's road to water scarcity: shortage and stress in the 20th century and pathways towards sustainability. Nat Publ Gr 6:1–16. doi: 10.1038/srep38495

Kutschera L, Lichtenegger E (1992) Wurzelatlas mitteleuropais- cher Griinlandpflanzen. Band 2: Pteridophyta und Dicotyle- doneae; Teil 1: Morphologie, Anatomie, Okologie, Verbreitung, Soziologie, Wirtschaft. Gustav Fischer, Stuttgart

Lambers H, Atkin OK, Millenaar FF (2000) Respiratory Patterns in Roots in Relation to Their Functioning. In: Plant Roots. The Hidden Half (Y. Waisel, A. Eshel, and K. Kafkaki, Eds.). Marcel Dekker, Inc. New York

Landsberg JJ, Fowkes ND (1978) Water Movement Through Plant Roots. Ann Bot 42:493–508. doi: 10.1093/oxfordjournals.aob.a085488

Lawes R, Renton M (2010) The Land Use Sequence Optimiser (LUSO): A theoretical framework for analysing crop sequences in response to nitrogen, disease and weed populations. Crop Pasture Sci 61:835–843

Lersten NR (1997) Occurence of endodermis with casparian strip in stem and leaf. Bot Rev

63:265–272

Li Y, Huang M (2008) Pasture yield and soil water depletion of continuous growing alfalfa in the Loess Plateau of China. Agric Ecosyst Environ 124:24–32. doi: 10.1016/j.agee.2007.08.007

Lian H, Yu X, Ye Q, et al (2004) The Role of Aquaporin RWC3 in Drought Avoidance in Rice. Plant Cell Physiol 45:481–489

Lilley JM, Kirkegaard JA (2011) Benefits of increased soil exploration by wheat roots. F Crop Res 122:118–130. doi: 10.1016/j.fcr.2011.03.010

Lilley JM, Kirkegaard JA (2016) Farming system context drives the value of deep wheat roots in semi-arid environments. J Exp Bot 67:3665–3681. doi: 10.1093/jxb/erw093

Lipiec J, Horn R, Pietrusiewicz J, Siczek A (2012) Effects of soil compaction on root elongation and anatomy of different cereal plant species. Soil Tillage Res 121:74–81. doi: 10.1016/j.still.2012.01.013

Lobet G, Couvreur V, Meunier F, et al (2014) Plant Water Uptake in Drying Soils. Plant Physiol 164:1619–1627. doi: 10.1104/pp.113.233486

Long SP, Ort DR (2010) More than taking the heat: crops and global change. Curr Opin Plant Biol 13:241–248. doi: 10.1016/j.pbi.2010.04.008

Lovisolo C, Schubert A (1998) Effects of water stress on vessel size and xylem hydraulic conductivity in Vitis vinifera L. J Exp Bot 49:693–700

Luu D, Maurel C (2013) Aquaporin Trafficking in Plant Cells: An Emerging Membrane-Protein Model. Traffic 14:629–635. doi: 10.1111/tra.12062

Lux A, Luxová M, Abe J (2004) Root cortex: Structural and functional variability and responses to environmental stress. Root Res 13:117–131. doi: 10.3117/rootres.13.117

Luxová M (1989) The vascular system in the roots of barley and its hydraulic aspects. In: Structural and functional aspects of transport in roots. pp 15–20

Luxová M (1990) Effect of Lateral Root Formation on the Vascular Pattern of Barley Roots. Bot Acta 103:305–310

Lynch JP (1995) Root Architecture and Plant Productivity. Plant Physiol 109:7–13

Lynch JP (2013) Steep, cheap and deep: An ideotype to optimize water and N acquisition by maize root systems. Ann Bot 112:347–357. doi: 10.1093/aob/mcs293

Lynch JP (2007a) TURNER REVIEW No. 14. Roots of the Second Green Revolution. Aust J Bot 55:493–512

Lynch JP (2019) Tansley review. Root phenotypes for improved nutrient capture: an underexploited opportunity for global agriculture. New Phytol 223:548–564. doi: 10.1111/nph.15738

Lynch JP (2007b) Rhizoeconomics: The Roots of Shoot Growth Limitations. HortScience 42:1107–1109

Lynch JP (2015) Root phenes that reduce the metabolic costs of soil exploration: opportunities for 21st century agriculture. Plant Cell Environ 38:1775–1784. doi: 10.1111/pce.12451

Lynch JP (2018) Rightsizing root phenotypes for drought resistance. J Exp Bot 69:3279–3292. doi: 10.1093/jxb/ery048

Lynch JP, Brown KM (2012) New roots for agriculture: exploiting the root phenome. Philos Trans R Soc B 367:1598–1604. doi: 10.1098/rstb.2011.0243

Lynch JP, Chimungu JG, Brown KM (2014) Root anatomical phenes associated with water acquisition from drying soil: Targets for crop improvement. J Exp Bot 65:6155–6166. doi: 10.1093/jxb/eru162

Lynch JP, Wojciechowski T (2015) Opportunities and challenges in the subsoil: Pathways to deeper rooted crops. J Exp Bot 66:2199–2210. doi: 10.1093/jxb/eru508

Lyu Y, Tang H, Li H, et al (2016) Major Crop Species Show Differential Balance between Root Morphological and Physiological Responses to Variable Phosphorus Supply. Front Plant Sci 7:1–15. doi: 10.3389/fpls.2016.01939

Macduff JH, Wild A, Hopper MJ, Dhanoa MS (1986) Effects of temperature on parameters of root growth relevant to nutrient uptake: Measurements on oilseed rape and barley grown in flowing nutrient solution. Plant Soil 94:321–332

Maeght J-L, Gonkhamdee S, Clément C, et al (2015) Seasonal Patterns of Fine Root Production and Turnover in a Mature Rubber Tree (Hevea brasiliensis Müll. Arg.) Stand- Differentiation with Soil Depth and Implications for Soil Carbon Stocks. Front Plant Sci 6:1–11. doi: 10.3389/fpls.2015.01022

Maeght J-L, Rewald B, Pierret A (2013) How to study deep roots-and why it matters. Front Plant Sci 4:299. doi: 10.3389/fpls.2013.00299

Mano Y, Omori F (2013) Relationship between constitutive root aerenchyma formation and flooding tolerance in Zea nicaraguensis. Plant Soil 370:447–460. doi: 10.1007/s11104-013-1641-0

Manschadi AM, Christopher J, Devoil P, Hammer GL (2006) The role of root architectural traits in adaptation of wheat to water-limited environments. Funct Plant Biol 33:823–837. doi: 10.1071/FP06055

Marquardt DW (1963) An algorithm for least-sqaures estimation of nonlinear parameters. J Soc Ind Appl Math 11:431–441

Marquardt K, Vico G, Glynn C, et al (2016) Farmer perspectives on introducing perennial cereal in Swedish farming systems: a sustainability analysis of plant traits, farm management, and ecological implications. Agroecol Sustain Food Syst 40:432–450. doi: 10.1080/21683565.2016.1141146

Martre P, North GB, Nobel PS (2001) Hydraulic Conductance and Mercury-Sensitive Water Transport for Roots of Opuntia acanthocarpa in Relation to Soil Drying and Rewetting. Plant Physiol 126:352–362

Martre P, North GB, Nobel PS, Chrispeels MJ (2002) Plasma Membrane Aquaporins Play a Significant Role during Recovery from Water Deficit. Plant Physiol 130:2101–2110. doi: 10.1104/pp.009019.which

Marzec M, Melzer M, Szarejko I (2015) Root Hair Development in the Grasses: What We Already Know and What We Still Need to Know. Plant Physiol 168:407–414. doi: 10.1104/pp.15.00158

Materechera SA, Alston AM, Kirby JM, Dexter AR (1992) Influence of root diameter on the penetration of seminal roots into a compacted subsoil. Plant Soil 144:297–303

Maurel C (1997) Aquaporins and water permeability of plant membranes. Annu Rev Plant Physiol Plant Mol Biol 48:399–429

Maurel C, Simonneau T, Sutka M (2010) The significance of roots as hydraulic rheostats. J Exp Bot 61:3191–3198. doi: 10.1093/jxb/erq150

Maurel C, Verdoucq L, Luu D (2008) Plant Aquaporins: Membrane Channels with Multiple Integrated Functions. Annu Rev Plant Biol 59:595–624. doi: 10.1146/annurev.arplant.59.032607.092734

Mazzacavallo MG, Kulmatiski A (2015) Modelling Water Uptake Provides a New Perspective on Grass and Tree Coexistence. PLoS One 10:1–16. doi: 10.1371/journal.pone.0144300

McCallum MH, Connor DJ, O'Leary GJ (2001) Water use by lucerne and effect on crops in the Victorian Wimmera. Aust J Agric Res 52:193–201. doi: 10.1071/AR99164

McCormack ML, Dickie IA, Eissenstat DM, et al (2015) Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytol 207:505–518

McCormack ML, Guo D (2014) Impacts of environmental factors on fine root lifespan. Front Plant Sci 5:1–11. doi: 10.1016/S1001-0742(09)60337-5

McCully ME (1995) How Do Real Roots Work? Some New Views of Root Structure. Plant Physiol 109:1–6

McCully ME (1999) ROOTS IN SOIL: Unearthing the Complexities of Roots and Their Rhizospheres. Annu Rev Plant Physiol Plant Mol Biol 50:695–718

McCully ME, Boyer JS (1997) The expansion of maize root-cap mucilage during hydration. 3. Changes in water potential and water content. Physiol Plant 99:169–178

McDaniel MD, Tiemann LK, Grandy AS (2014) Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecol Appl 24:560–570

McElrone AJ, Bichler J, Pockman WT, et al (2007) Aquaporin-mediated changes in hydraulic conductivity of deep tree roots accessed via caves. Plant Cell Environ 30:1411–1421. doi: 10.1111/j.1365-3040.2007.01714.x

McElrone AJ, Choat B, Gambetta GA, Brodersen CR (2013) From the Soil into the Plant. Nat Educ Knowl 4:5–6

McElrone AJ, Pockman WT, Martínez-vilalta J, Jackson RB (2004) Variation in xylem structure and function in stems and roots of trees to 20 m depth. New Phytol 163:507–517

McIntosh MS, Miller DA (1980) Development of root-branching in three alfalfa cultivars. Crop Sci 20:807–809

McIsaac GF, David MB, Mitchell CA (2010) Miscanthus and Switchgrass Production in Central Illinois: Impacts on Hydrology and Inorganic Nitrogen Leaching. J Environ Qual 39:1790–1799. doi: 10.2134/jeq2009.0497

Mclean EH, Ludwig M, Grierson PF (2011) Root hydraulic conductance and aquaporin abundance respond rapidly to partial root-zone drying events in a riparian Melaleuca species. New Phytol 192:664–675

Meyer RS, Duval AE, Jensen HR (2012) Tansley review. Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. New Phytol 196:29–48

Mikhailova EA, Bryant RB, Vassenev II, et al (2000) Cultivation Effects on Soil Carbon and Nitrogen Contents at Depth in the Russian Chernozem. Soil Sci Soc Am J 64:738–745. doi: 10.2136/sssaj2000.642738x

Mikhailova EA, Post CJ (2006) Organic carbon stocks in the Russian Chernozem. Eur J Soil Sci 57:330–336. doi: 10.1111/j.1365-2389.2005.00741.x

Mitchell RB, Moser LE, Moore KJ, Redfearn DD (1998) Tiller Demographics and Leaf Area Index of Four Perennial Pasture Grasses. Agron J 90:47–53. doi: 10.2134/agronj1998.00021962009000010009x

Molden D, Oweis T, Steduto P, et al (2010) Improving agricultural water productivity: Between optimism and caution. Agric Water Manag 97:528–535. doi: 10.1016/j.agwat.2009.03.023

Monti A, Zatta A (2009) Root distribution and soil moisture retrieval in perennial and annual energy crops in Northern Italy. Agric Ecosyst Environ 132:252–259. doi: 10.1016/j.agee.2009.04.007

Muller B, Guédon Y, Passot S, et al (2019) Lateral Roots: Random Diversity in Adversity. Trends Plant Sci 24:810–825. doi: 10.1016/j.tplants.2019.05.011

Munns R, Tester M (2008) Mechanisms of Salinity Tolerance. Annu Rev Plant Biol 59:651–81. doi: 10.1146/annurev.arplant.59.032607.092911

Nagel KA, Kastenholz B, Jahnke S, et al (2009) Temperature responses of roots: impact on growth, root system architecture and implications for phenotyping. Funct Plant Biol 36:947–959

Nestler J, Keyes SD, Wissuwa M (2016) Root hair formation in rice (Oryza sativa L.) differs between root types and is altered in artificial growth conditions. J Exp Bot 67:3699–3708. doi: 10.1093/jxb/erw115

Nielsen MH, Styczen M, Ernstsen V, et al (2010) Field Study of Preferential Flow Pathways in and between Drain Trenches. Vadose Zo J 9:1073–1079. doi: 10.2136/vzj2010.0013.

Nobel P (2009) Physicochemical and Environmental Plant Physiology

Orians CM, Babst B, Zanne AE (2005) Vascular Constraints and Long Distance Transport in Dicots. In: Vascular Transport in Plants. pp 355–371

Parent B, Hachez C, Redondo E, et al (2009) Drought and Abscisic Acid Effects on Aquaporin Content Translate into Changes in Hydraulic Conductivity and Leaf Growth Rate: A Trans-Scale Approach. Plant Physiol 149:2000–2012. doi: 10.1104/pp.108.130682

Passioura JB (1983) Roots and drought resistance. Agric Water Manag 7:265–280. doi: 10.1016/0378-3774(83)90089-6

Passioura JB (1991) Soil Structure and Plant-Growth. Aust J Soil Res 29:717–728. doi: 10.1071/SR9910717

Passioura JB (2020) Translational research in agriculture. Can we do it better? Crop Pasture Sci

71:517–528. doi: 10.1071/CP20066

Passioura JB (2006) Increasing crop productivity when water is scarce - From breeding to field management. Agric Water Manag 80:176–196. doi: 10.1016/j.agwat.2005.07.012

Passioura JB, Angus JF (2010) Improving Productivity of Crops in Water-Limited Environments, 1st edn.

Pate JS, Jeschke WD, Aylward MJ (1995) Hydraulic architecture and xylem structure of the dimorphic root systems of South-West Australian species of Proteaceae. J Exp Bot 46:907–915

Peterson CA, Enstone DE (1996) Functions of passage cells in the endodermis and exodermis of roots. Physiol Plant 97:592–598

Peterson CA, Steudle E (1993) Lateral hydraulic conductivity of early metaxylem vessels in Zea mays L. roots. Planta 189:288–297. doi: 10.1007/BF00195088

Pierret A, Doussan C, Pagès L (2006) Spatio-temporal variations in axial conductance of primary and first-order lateral roots of a maize crop as predicted by a model of the hydraulic architecture of root systems. Plant Soil 282:117–126. doi: 10.1007/s11104-005-5373-7

Pierret A, Maeght J-L, Clément C, et al (2016) Understanding deep roots and their functions in ecosystems: an advocacy for more unconventional research. Ann Bot 118:1–15. doi: 10.1093/aob/mcw130

Pietola LM, Smucker AJM (1995) Fine Root Dynamics of Alfalfa after Multiple Cuttings and During a Late Invasion by Weeds. Agron J 87:1161–1169. doi: 10.2134/agronj1995.00021962008700060021x

Pimentel D, Cerasale D, Stanley RC, et al (2012) Annual vs. perennial grain production. Agric Ecosyst Environ 161:1–9. doi: 10.1016/j.agee.2012.05.025

Pinheiro HA, DaMatta FM, Chaves ARM, et al (2005) Drought Tolerance is Associated with Rooting Depth and Stomatal Control of Water Use in Clones of Coffea canephora. Ann Bot 96:101–108. doi: 10.1093/aob/mci154

Poca M, Coomans O, Urcelay C (2019) Isotope fractionation during root water uptake by Acacia caven is enhanced by arbuscular mycorrhizas. Plant Soil 441:485–497. doi: 10.1007/s11104-019-04139-1

Poorter H, Bühler J, Van Dusschoten D, et al (2012) Pot size matters : A meta-analysis of the effects of rooting volume on plant growth. Funct Plant Biol 39:839–850. doi: 10.1071/FP12049

Poorter H, Fiorani F, Pieruschka R, et al (2016) Pampered inside, pestered outside? Differences and similarities between plants growing in controlled conditions and in the field. New Phytol 212:838–855

Prado K, Maurel C (2013) Regulation of leaf hydraulics: from molecular to whole plant levels parameter. Front Plant Sci 4:1–14. doi: 10.3389/fpls.2013.00255

Prechsl UE, Burri S, Gilgen AK, et al (2015) No shift to a deeper water uptake depth in response to summer drought of two lowland and sub-alpine C3-grasslands in Switzerland. Oecologia 177:97–111. doi: 10.1007/s00442-014-3092-6

Prince SJ, Murphy M, Mutava RN, et al (2018) Root xylem plasticity to improve water use and yield in water-stressed soybean. J Exp Bot 68:2027–2036. doi: 10.1093/jxb/erw472

Pugliese JY (2017) Above- and Belowground Response to Managing Kernza (Thinopyrum intermedium) as a Dual-Use Crop for Forage and Grain (Master's thesis)

Pugliese JY, Culman SW, Sprunger CD (2019) Harvesting forage of the perennial grain crop kernza (Thinopyrum intermedium) increases root biomass and soil nitrogen. Plant Soil 437:241–254

Purushothaman R, Zaman-allah M, Mallikarjuna N, et al (2013) Root Anatomical Traits and Their Possible Contribution to Drought Tolerance in Grain Legumes. Plant Prod Sci 16:1–8

Randall GW, Mulla DJ (2001) Nitrate Nitrogen in Surface Waters as Influenced by Climatic Conditions and Agricultural Practices. J Environ Qual 30:337–344

Rasmussen CR, Thorup-Kristensen K, Dresbøll DB (2019) Uptake of subsoil water below 2 m fails to alleviate drought response in deep-rooted Chicory (Cichorium intybus L.). Plant Soil

446:275–290

Rasmussen SB, Blenkinsop S, Burton A, et al (2018) Climate change impacts on agro-climatic indices derived from downscaled weather generator scenarios for eastern Denmark. Eur J Agron 101:222–238. doi: 10.1016/J.EJA.2018.04.004

Rasse DP, Smucker AJM, Santos D (2000) Alfalfa Root and Shoot Mulching Effects on Soil Hydraulic Properties and Aggregation. Soil Sci Soc Am J 64:725–731. doi: 10.2136/sssaj2000.642725x

Raun WR, Johnson G V. (1999) Improving Nitrogen Use Efficiency for Cereal Production. Agron J 91:357–363

Ray DK, Mueller ND, West PC, Foley JA (2013) Yield Trends Are Insufficient to Double Global Crop Production by 2050. PLoS One 8:e66428. doi: 10.1371/journal.pone.0066428

Reissinger A, Winter S, Steckelbroeck S, et al (2003) Infection of barley roots by Chaetomium globosum: evidence for a protective role of the exodermis. Mycol Res 107:1094–1102. doi: 10.1017/S0953756203008189

Richards RA, Passioura JB (1981a) Seminal Root Morphology and Water Use of Wheat I. Environmental Effects. Crop Sci 21:249–252. doi: 10.2135/cropsci1981.0011183X002100020011x

Richards RA, Passioura JB (1981b) Seminal Root Morphology and Water Use of Wheat II. Genetic Variation. Crop Sci 21:253–255. doi: 10.2135/cropsci1981.0011183X002100020012x

Richards RA, Passioura JB (1989) A Breeding Program to Reduce the Diameter of the Major Xylem Vessel in the Seminal Roots of Wheat and its Effect on Grain Yield in Rain-fed Environments. Aust J Agric Res 40:943–950

Riday H, Brummer EC (2002) Forage Yield Heterosis in Alfalfa. Crop Sci 42:716–723

Robards AW, Clarkson DT (1976) The role of plasmodesmata in the transport of water and nutrients across roots. In: Intercellular communication in plants: Studies on plasmodesmata. pp 181–201

Roberts A, Kingdon U, Oparka K (2003) Plasmodesmata and the control of symplastic transport. Plant Cell Environ 26:103–124. doi: 10.1046/j.1365-3040.2003.00950.x

Rosegrant MW, Ringler C, Zhu T (2009) Water for Agriculture: Maintaining Food Security under Growing Scarcity. Annu Rev Environ Resour 34:205–222. doi: 10.1146/annurev.environ.030308.090351

Rothfuss Y, Javaux M (2017a) Suppl. Isotopic approaches to quantifying root water uptake and redistribution: a review and comparison of methods. Biogeosciences Discuss 1–47. doi: 10.5194/bg-2016-410

Rothfuss Y, Javaux M (2017b) Reviews and syntheses: Isotopic approaches to quantify root water uptake: A review and comparison of methods. Biogeosciences 14:2199–2224. doi: 10.5194/bg-14-2199-2017

Ryan PR, Delhaize E, Watt M, Richardson AE (2016) Plant roots: Understanding structure and function in an ocean of complexity. Ann Bot 118:555–559. doi: 10.1093/aob/mcw192

Sack L, Holbrook NM (2006) Leaf Hydraulics. Annu Rev Plant Physiol Plant Mol Biol 57:361–81. doi: 10.1146/annurev.arplant.56.032604.144141

Sade N, Gebremedhin A, Moshelion M (2012) Risk-taking plants: Anisohydric behavior as a stress-resistance trait. Plant sign 7:767–770. doi: 10.4161/psb.20505

Sade N, Vinocur BJ, Diber A, et al (2009) Improving plant stress tolerance and yield production: is the tonoplast aquaporin SlTIP2;2 a key to isohydric to anisohydric conversion? New Phytol 181:651–661

Saengwilai P, Nord EA, Chimungu JG, et al (2014) Root Cortical Aerenchyma Enhances Nitrogen Acquisition from Low-Nitrogen Soils in Maize. Plant Physiol 166:726–735. doi: 10.1104/pp.114.241711

Sainju UM, Allen BL, Lenssen AW, Ghimire RP (2017) Root biomass, root/shoot ratio, and soil water content under perennial grasses with different nitrogen rates. F Crop Res 210:183–191. doi: 10.1016/j.fcr.2017.05.029

Sakurai-Ishikawa J, Murai-Hatano M, Hayashi H, et al (2011) Transpiration from shoots triggers diurnal changes in root aquaporin expression. Plant Cell Environ 34:1150–1163. doi: 10.1111/j.1365-3040.2011.02313.x

Sanderson J (1983) Water uptake by different regions of the barley root pathway of radial flow in relation to development of the endodermis. J Exp Bot 34:240–253

Schenk HJ, Jackson RB (2002) The global biogeography of roots. Ecol Monogr 72:311–328. doi: 10.1890/0012-9615(2002)072[0311:TGBOR]2.0.CO;2

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675. doi: 10.1038/nmeth.2089

Schneider HM, Lynch JP (2017) Functional implications of root cortical senescence for soil resource capture. Plant Soil 423:13–26. doi: 10.1007/s11104-017-3533-1

Schneider HM, Wojciechowski T, Postma JA, et al (2017) Root cortical senescence decreases root respiration, nutrient content and radial water and nutrient transport in barley. Plant Cell Environ 40:1392–1408. doi: 10.1111/pce.12933

Schnepf A, Black CK, Couvreur, Valentin, Delory BM, et al (2020) Call for Participation: Collaborative Benchmarking of Functional-Structural Root Architecture Models. The Case of Root Water Uptake. Front Plant Sci 11:1–21. doi: 10.3389/fpls.2020.00316

Schoppach R, Wauthelet D, Jeanguenin L, Sadok W (2013) Conservative water use under high evaporative demand associated with smaller root metaxylem and limited trans-membrane water transport in wheat. Funct Plant Biol 41:257–269. doi: 10.1071/FP13211

Schreiber L, Franke RB (2011) Endodermis and Exodermis in Roots. Encycl Life Sci 1–7. doi: 10.1002/9780470015902.a0002086.pub2

Segal E, Kushnir T, Mualem Y, Shani U (2008) Water Uptake and Hydraulics of the Root Hair Rhizosphere. Vadose 7:1027–1034. doi: 10.2136/vzj2007.0122

Sellin A (1993) Resistance to water flow in xylem of Picea abies (L.) Karst. trees grown under contrasting light conditions. Trees 7:220–226

Seta AK, Blevins RL, Frye WW, Barfield BJ (1993) Reducing Soil Erosion and Agricultural Chemical Losses with Conservation Tillage. J Environ Qual 22:661–665

Sharp RE, Davies WJ (1985) Root Growth and Water Uptake by Maize Plants in Drying Soil. J Exp Bot 36:1441–1456

Sheaffer CC, Tanner CB, Kirkham MB (1988) Alfalfa water relations and irrigation. In: Alfalfa and Alfalfa Improvement-Agronomy Monograph no. 29.

Simonds ONA (1932) Histological studies of the development of the root and crown of alfalfa (PhD Thesis)

Simunek J, Hopmans JW (2009) Modeling compensated root water and nutrient uptake. Ecol Modell 220:505–521. doi: 10.1016/j.ecolmodel.2008.11.004

Šimůnek J, Šejna M, Saito H, et al (2009) The HYDRUS-1D Software Package for Simulating the One-Dimensional Movement of Water, Heat, and Multiple Solutes in Variably-Saturated Media

Skaggs TH, van Genuchten MT, Shouse PJ, Poss JA (2006) Macroscopic approaches to root water uptake as a function of water and salinity stress. Agric Water Manag 86:140–149. doi: 10.1016/j.agwat.2006.06.005

Smith AG, Han E, Petersen J, et al (2020) RootPainter: Deep Learning Segmentation of Biological Images with Corrective Annotation. BioRxiv 1–16. doi: 10.1101/2020.04.16.044461

Sprunger CD, Culman SW, Robertson GP, Snapp SS (2018) How Does Nitrogen and Perenniality Influence Belowground Biomass and Nitrogen Use Efficiency in Small Grain Cereals? Crop Sci 58:1–11. doi: 10.2135/cropsci2018.02.0123

Steudle E (2000a) Water uptake by roots: an integration of views. Plant Soil 226:45–56

Steudle E (2000b) Water uptake by roots: effects of water deficit. J Exp Bot 51:1531–1542. doi: 10.1093/jexbot/51.350.1531

Steudle E, Frensch J (1996) Water transport in plants: Role of the apoplast. Plant Soil 187:67–79

Steudle E, Peterson CA (1998) How does water get through roots? J Exp Bot 49:775–788. doi: 10.1093/jxb/49.322.775

Stirzaker RJ, Passioura JB (1996) The water relations of the root–soil interface. Plant Cell Environ 19:201–208. doi: 10.1111/j.1365-3040.1996.tb00241.x

Striker GG (2012) Flooding Stress on Plants: Anatomical, Morphological and Physiological Responses. In: Botany, Dr. John Mworia (Ed.)

Strock CF, Lynch JP (2020) Root secondary growth: An unexplored component of soil resource acquisition. Ann Bot 126:205–218. doi: doi.org/10.1093/aob/mcaa068

Strock CF, Schneider HM, Galindo-castañeda T, et al (2019) Laser ablation tomography for visualization of root colonization by edaphic organisms. J Exp Bot 70:5327–5342. doi: 10.1093/jxb/erz271

Sutanto SJ, Wenninger J, Coenders-Gerrits, AMJ, Uhlenbrook S (2012) Partitioning of evaporation into transpiration, soil evaporation and interception: a comparison between isotope measurements and a HYDRUS-1D model. Hydrol Earth Syst Sci 16:2605–2616. doi: 10.5194/hess-16-2605-2012

Svane SF, Dam EB, Carstensen JM, Thorup-Kristensen K (2019a) A multispectral camera system for automated minirhizotron image analysis. Plant Soil 441:657–672. doi: 10.1007/s11104-019-04132-8

Svane SF, Jensen CS, Thorup-Kristensen K (2019b) Construction of a large-scale semi-field facility to study genotypic differences in deep root growth and resources acquisition. Plant Methods 15:1–16. doi: 10.1186/s13007-019-0409-9

Svane SF, Svensgaard J, Petersen CT (2020) Meteorological data from the Taastrup Climate and Water Balance Station 2014-2019. In: https://doi.org/10.17894/ucph.8d941a14-b098-4ca5-b177-412f50be1731

Takase T, Ishikawa H, Murakami H, et al (2011) The Circadian Clock Modulates Water Dynamics and Aquaporin Expression in Arabidopsis Roots. Plant Cell Physiol 52:373–383. doi: 10.1093/pcp/pcq198

Tardieu F, Davies WJ (1983) Integration of hydraulic and chemical signalling in the control of stomatal conductance and water status of droughted plants. Plant Cell Environ 16:341–349

Tardieu F, Simonneau T (1998) Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours. J Exp Bot 49:419–432. doi: 10.1093/jexbot/49.suppl

Taylor SA, Asshcroft GL (1972) Physical edaphology. The physics of irrigated and nonirrigated soils

Tester M, Leigh RA (2001) Partitioning of nutrient transport processes in roots. J Exp Bot 52:445–457

Thomas A, Yadav BK, Šimůnek J (2020) Root water uptake under heterogeneous soil moisture conditions: an experimental study for unraveling compensatory root water uptake and hydraulic redistribution. Plant Soil 457:421–435. doi: 10.1007/s11104-020-04738-3

Thorup-Kristensen K (2001) Are differences in root growth of nitrogen catch crops important for their ability to reduce soil nitrate-N content, and how can this be measured? Plant Soil 230:185–195. doi: 10.1023/A:1010306425468

Thorup-Kristensen K (2006) Effect of deep and shallow root systems on the dynamics of soil inorganic N during 3-year crop rotations. Plant Soil 288:233–248. doi: 10.1007/s11104-006-9110-7

Thorup-Kristensen K, Cortasa MS, Loges R (2009) Winter wheat roots grow twice as deep as spring wheat roots, is this important for N uptake and N leaching losses ? Plant Soil 322:101–114. doi: 10.1007/s11104-009-9898-z

Thorup-Kristensen K, Dresbøll DB, Kristensen HL (2012) Crop yield, root growth, and nutrient dynamics in a conventional and three organic cropping systems with different levels of external inputs and N re-cycling through fertility building crops. Eur J Agron 37:66–82. doi: 10.1016/j.eja.2011.11.004

Thorup-Kristensen K, Halberg N, Nicolaisen M, et al (2020a) Digging Deeper for Agricultural Resources, the Value of Deep Rooting. Trends Plant Sci 25:406–417. doi: 10.1016/j.tplants.2019.12.007

Thorup-Kristensen K, Halberg N, Nicolaisen MH, et al (2020b) Exposing Deep Roots: A Rhizobox Laboratory. Trends Plant Sci 25:1–2. doi: 10.1016/j.tplants.2019.12.006

Thorup-Kristensen K, Kirkegaard J (2016) Root system-based limits to agricultural productivity and efficiency: the farming systems context. Ann Bot 118:573–592. doi: 10.1093/aob/mcw122

Thorup-Kristensen K, Rasmussen CR (2015) Identifying new deep-rooted plant species suitable as undersown nitrogen catch crops. J Soil Water Conserv 70:399–409. doi: 10.2489/jswc.70.6.399

Tilman D, Cassman KG, Matson PA, et al (2002) Agricultural sustainability and intensive production practices. Nature 418:671–677. doi: 10.1038/nature01014

Tonn N, Greb T (2017) Radial plant growth. Curr Biol 27:878–882. doi: 10.1016/j.cub.2017.03.056

Toreti A, Belward A, Perez-dominguez I, Naumann G (2019) The Exceptional 2018 European Water Seesaw Calls for Action on Adaptation. Earth’s Futur 7:652–663

Tournaire-Roux C, Sutka M, Javot H, et al (2003) Cytosolic pH regulates root water transport during anoxic stress through gating of aquaporins. Nature 425:393–397

Turral H, Burke J, Faures JM, Faures JM (2011) Climate change, water and food security. Rome Food Agric Organ United Nations 204

Tyerman SD, Niemietz CM, Bramley H (2002) Plant aquaporins: multifunctional water and solute channels. Plant Cell Environ 25:173–194

Tylová E, Pecková E, Blascheová Z, Ales S (2017) Casparian bands and suberin lamellae in exodermis of lateral roots: an important trait of roots system response to abiotic stress factors. Ann Bot 120:71–85. doi: 10.1093/aob/mcx047

Tyree MT, Sperry JS (1989) Vulnerability of xylem to cavitation and embolism. Annu Rev Plant Physiol Plant Mol Biol 40:19–38

Ueda S (1936) On the development of root system of wheat and barley in sandy soil. Crop Sci Soc Jpn 8:140–143

Uga Y, Sugimoto K, Ogawa S, et al (2013) Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nat Genet 45:1097–1105. doi: 10.1038/ng.2725

Vadez V (2014) Root hydraulics: The forgotten side of roots in drought adaptation. F Crop Res 165:15–24. doi: 10.1016/j.fcr.2014.03.017

Vadez V, Kholova J, Medina S, et al (2014) Transpiration efficiency: new insights into an old story. J Exp Bot 65:6141–6153. doi: 10.1093/jxb/eru040

Valentine TA, Hallett PD, Binnie K, et al (2012) Soil strength and macropore volume limit root elongation rates in many UK agricultural soils. Ann Bot 110:259–270. doi: 10.1093/aob/mcs118

Van Den Honert T. (1948) Water transport in plants as a catenary process. Discuss Faraday Soc 3:146–153

Van Der Krift TAJ, Berendse F (2002) Root life spans of four grass species from habitats. Funct Ecol 16:198–203

Van Genuchten MT (1980) A closed-form equation for predicting the hydraulic conductivity of unsaturated soils. Soil Sci Soc Am J 44:892–898. doi: 10.2136/sssaj1980.03615995004400050002x

Vandeleur RK, Mayo G, Shelden MC, et al (2009) The Role of Plasma Membrane Intrinsic Protein Aquaporins in Water Transport through Roots: Diurnal and Drought Stress Responses Reveal Different Strategies between Isohydric and Anisohydric Cultivars. Plant Physiol 149:445–460. doi: 10.1104/pp.108.128645

Vandeleur RK, Sullivan W, Athman A, et al (2014) Rapid shoot-to-root signalling regulates root hydraulic conductance via aquaporins. Plant, Cell Environ 37:520–538. doi: 10.1111/pce.12175

Vandoorne B, Beff L, Lutt S, Javaux M (2012) Root Water Uptake Dynamics of Cichorium intybus var. sativum Under Water-Limited Conditions. Vadoze Zo J 11:1–16. doi:

10.2136/vzj2012.0005

Vargas AI, Schaffer B, Yuhong L, da Silveira Lobo Sternberg L (2017) Testing plant use of mobile vs immobile soil water sources using stable isotope experiments. New Phytol 215:582–594. doi: 10.1111/nph.14616

Vartapetian BB, Jackson MB (2015) Plant adaptations to anaerobic stress. Ann Bot 79:3–20. doi: 10.1093/oxfordjournals.aob.a010303

Vercambre G, Doussan C, Pages L, et al (2002) Influence of xylem development on axial hydraulic conductance within Prunus root systems. Trees 16:479–487. doi: 10.1007/s00468-002-0190-6

Vico G, Brunsell NA (2018) Tradeoffs between water requirements and yield stability in annual vs. perennial crops. Adv Water Resour 112:189–202. doi: 10.1016/j.advwatres.2017.12.014

Wagoner P (1990) Perennial grain: New use for intermediate wheatgrass. J Soil Water Conserv 45:81–82

Wahlström EM, Hansen EM, Mandel A, et al (2015) Root development of fodder radish and winter wheat before winter in relation to uptake of nitrogen. Eur J Agron 71:1–9. doi: 10.1016/j.eja.2015.07.002

Walter A, Silk WK, Schurr U (2009) Environmental Effects on Spatial and Temporal Patterns of Leaf and Root Growth. Annu Rev Plant Biol 60:279–304. doi: 10.1146/annurev.arplant.59.032607.092819

Wang Y, Dong X, Wang H, et al (2015) Root tip morphology, anatomy, chemistry and potential hydraulic conductivity vary with soil depth in three temperate hardwood species. Tree Physiol 36:99–108. doi: 10.1093/treephys/tpv094

Wang Y, Zhang Y, Ji W, et al (2016) Cultivar Mixture Cropping Increased Water Use Efficiency in Winter Wheat under Limited Irrigation Conditions. PLoS One 11:1–15. doi: 10.1371/journal.pone.0158439

Ward PR, Dunin FX, Micin SF (2002) Water use and root growth by annual and perennial pastures and subsequent crops in a phase rotation. Agric Water Manag 53:83–97

Ward PR, Palta JA, Waddell HA (2011) Root and shoot growth by seedlings of annual and perennial medic, and annual and perennial wheat. Crop Pasture Sci 62:367–373. doi: 10.1071/CP10392

Wasson AP, Richards RA, Chatrath R, et al (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. J Exp Bot 63:3485–3498. doi: 10.1093/jxb/ers111

Watanabe K, Nishiuchi S, Kulichikhin K, Nakazono M (2013) Does suberin accumulation in plant roots contribute to waterlogging tolerance ? Front Plant Sci 4:1–7. doi: 10.3389/fpls.2013.00178

Watt M, Magee LJ, McCully ME (2008) Types, structure and potential for axial water flow in the deepest roots of field-grown cereals. New Phytol 178:135–146. doi: 10.1111/j.1469-8137.2007.02358.x

Watt M, McCully ME, Canny MJ (1994) Formation and Stabilization of Rhizosheaths of Zea mays. Effect of Soil Water Content. Plant Physiol 106:179–186

Weiner J (2017) Applying plant ecological knowledge to increase agricultural sustainability. J Ecol 105:865–870. doi: 10.1111/1365-2745.12792

Weraduwage SM, Chen J, Anozie FC, et al (2015) The relationship between leaf area growth and biomass accumulation in Arabidopsis thaliana. Front Plant Sci 6:1–21. doi: 10.3389/fpls.2015.00167

Wesseling JG (1991) Meerjarige simulaties van grondwateronttrekking voor verschillende bodemprofielen, grondwatertrappen en gewassen met het model SWATRE. SC-DLO Rep 152 DLO Winand Star Ctr, Wa- geningen, Netherlands

White RG, Kirkegaard JA (2010) The distribution and abundance of wheat roots in a dense, structured subsoil - Implications for water uptake. Plant, Cell Environ 33:133–148. doi: 10.1111/j.1365-3040.2009.02059.x

Whitmore AP, Whalley WR (2009) Physical effects of soil drying on roots and crop growth. J Exp

Bot 60:2845–2857. doi: 10.1093/jxb/erp200

Wong MTF, Asseng S, Robertson MJ, Oliver Y (2008) Mapping subsoil acidity and shallow soil across a field with information from yield maps, geophysical sensing and the grower. Precis Agric 9:3–15. doi: 10.1007/s11119-008-9052-6

Xu C, Tao H, Tian B, et al (2016) Limited-irrigation improves water use efficiency and soil reservoir capacity through regulating root and canopy growth of winter wheat. F Crop Res 196:268–275. doi: 10.1016/j.fcr.2016.07.009

Yambao EB, Ingram KT, Real JG (1992) Root Xylem Influence on the Water Relations and Drought Resistance of Rice. J Exp Bot 43:925–932

York LM, Carminati A, Mooney SJ, et al (2017) The holistic rhizosphere: integrating zones, processes, and semantics in the soil influenced by roots. J Exp Bot 67:3629–3643. doi: 10.1093/jxb/erw108

York LM, Nord EA, Lynch JP (2013) Integration of root phenes for soil resource acquisition. Front Plant Sci 4:1–15. doi: 10.3389/fpls.2013.00355

Zarebanadkouki M, Ahmed M, Hedwig C, et al (2018) Rhizosphere hydrophobicity limits root water uptake after drying and subsequent rewetting. Plant Soil 428:265–277

Zarebanadkouki M, Kim YX, Carminati A (2013) Where do roots take up water? Neutron radiography of water flow into the roots of transpiring plants growing in soil. New Phytol 199:1034–1044. doi: 10.1111/nph.12330

Zarebanadkouki M, Meunier F, Couvreur V, et al (2016) Estimation of the hydraulic conductivities of lupine roots by inverse modelling of high-resolution measurements of root water uptake. Ann Bot 118:853–864. doi: 10.1093/aob/mcw154

Zhan A, Schneider H, Lynch JP (2015) Reduced Lateral Root Branching Density Improves Drought Tolerance in Maize. Plant Physiol 168:1603–1615. doi: 10.1104/pp.15.00187

Zhang X, Pei D, Chen S, et al (2006) Performance of Double-Cropped Winter Wheat–Summer Maize under Minimum Irrigation in the North China Plain. Agron J 98:1620–1626. doi: 10.2134/agronj2005.0358

Zheng L, Ma J, Sun X, et al (2018) Estimating the Root Water Uptake of Surface-Irrigated Apples Using Water Stable Isotopes and the Hydrus-1D Model. Water 10:1–12. doi: 10.3390/w10111624

Zhou LL, Cao J, Zhang FS, Li L (2009) Rhizosphere acidification of faba bean, soybean and maize. Sci Total Environ 407:4356–4362. doi: 10.1016/j.scitotenv.2009.02.006

Zhou S, Wu Y, Wang Z, et al (2008) The nitrate leached below maize root zone is available for deep-rooted wheat in winter wheat-summer maize rotation in the North China Plain. Environ Pollut 152:723–730. doi: 10.1016/j.envpol.2007.06.047

Zhu X, Long SP, Ort DR (2010) Improving Photosynthetic Efficiency for Greater Yield. Annu Rev Plant Biol 61:235–261. doi: 10.1146/annurev-arplant-042809-112206

Zimmermann HM, Hartmann K, Schreiber L, Steudle E (2000) Chemical composition of apoplastic transport barriers in relation to radial hydraulic conductivity of corn roots (Zea mays L.). Planta 210:302–311

Zook DM, Erwin DC, Stolzy LH (1986) Anatomical, morphological, and physiological responses of alfalfa to flooding. Plant Soil 96:293–296. doi: 10.1007/BF02374773

Zwieniecki MA, Thompson M V, Holbrook NM (2003) Understanding the Hydraulics of Porous Pipes: Tradeoffs Between Water Uptake and Root Length Utilization. J Plant Growth Regul 21:315–323. doi: 10.1007/s00344-003-0008-9