Spatiotemporal responses of wheat and pea roots to fluctuations in soil physical conditions

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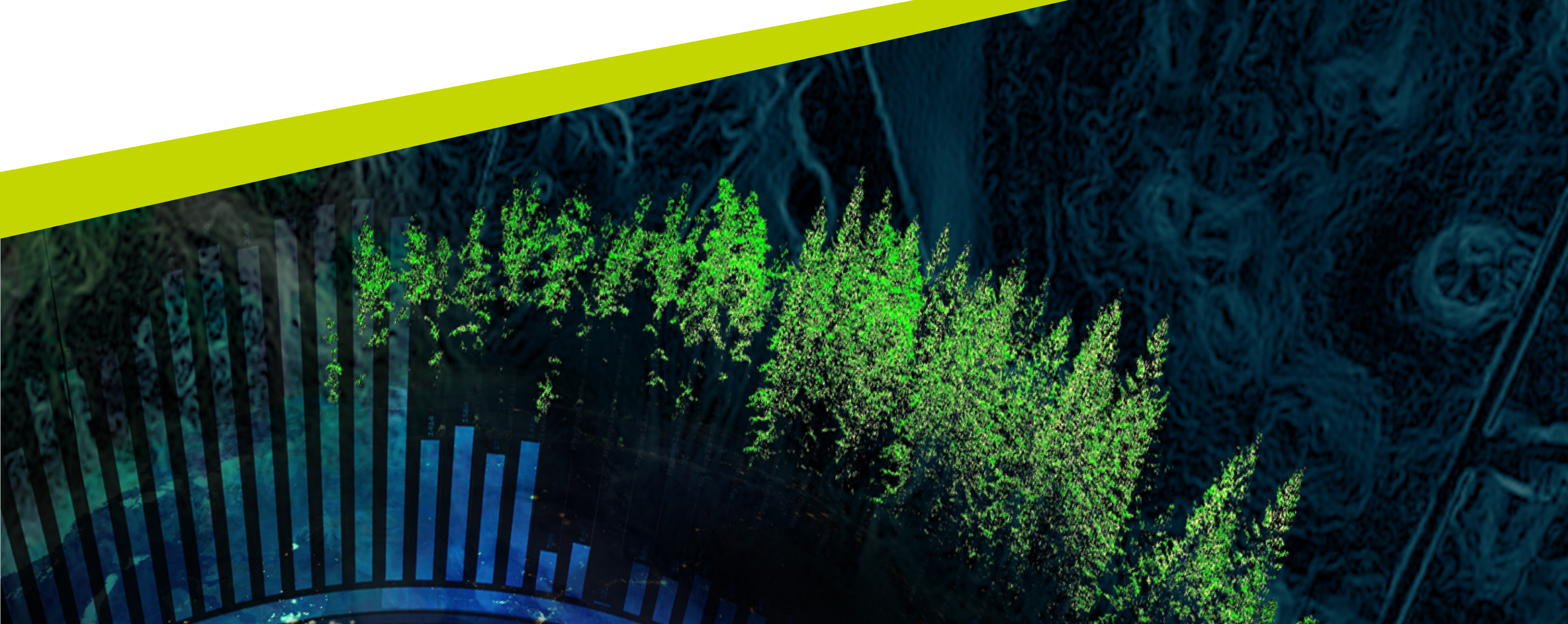

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Abstract

Penetration resistance and the oxygen level vary in the soil, and a root will face fluctuations in these physical properties during development. The aim of this study was to understand how pea (*Pisum sativum L.*) and wheat (*Triticum aestivum L.*) roots are affected in fluctuations of soil penetration resistance, the oxygen concentration in soil air and a combination of those.

Root growth rate and root diameter were quantified during six days of growth in rhizoboxes. Root growth was recorded in intervals of 20 minutes using automated time-lapse imaging and the diameter was measured in images from a flatbed scanner. The roots were subjected to increased penetration resistance, hypoxic conditions or a combination of those two times during the growing period, with a release of the physical stress in between.

Pea and wheat showed different but consistent patterns in root growth rate. Both pea and wheat responded with a decrease in root growth rate when subjected to physical stress. The difference was that pea had an increase, and recovered, in root growth rate upon the release of the stress, while wheat did not. Regarding the diameter, the results had an inconsistent pattern and did not fully correspond with previous studies.

The ability of pea roots to recover in root growth rate upon release of physical stress implies that pea roots can reach deeper in the soil than wheat roots after facing fluctuations in penetration resistance and oxygen concentration.

Populärvetenskaplig sammanfattning

Välmående jordar är livsnödvändigt för jordbruket och matproduktion. Ett ökande problem i och med växande världsbefolkning, och som hotar framtidens matproduktion, är försämrad jordstruktur. Två processer som försämrar jordstrukturen är ökad markpackning och vattenmättnad. De förekommer allt oftare på grund av mer intensiv jordbearbetning med tunga maskiner och klimatförändringar såsom ökad lokal nederbörd och översvämningar. I en kompakt jord ökar trycket som en rot behöver övervinna för att kunna växa, och i en vattenmättad jord förekommer syrebrist när porerna i marken blir vattenfyllda, vilka båda påverkar rottillväxten negativt. En konsekvens av försämrad tillväxt på rötterna är att mindre vatten och näringsämnen kan nås av rotsystemet, vilket påverkar plantan och på längre sikt skörden negativt.

Jorden bidrar till stöd för rötter och är en källa för näringsämnen, vatten och syre till plantan. Samtidigt påverkar rötterna jordstrukturen positivt genom att skapa regelbundna porer. I dessa porer kan luft och vatten transporteras och lagras, men även fungera som gångar som framtida rötter kan växa i. I en jord förekommer variationer med lokala områden med olika högt tryck eller syremängd. Rötter försöker anpassa sig till dessa varierande fysiska påfrestningar. Anpassningar för att klara av syrebrist är att skapa hålrum i roten där syre snabbt kan transporteras, skapa fler sidorötter eller att öka diametern på roten. För att klara av ett ökat tryck i marken kan rotspetsen utsöndra ett sekret eller offra de yttersta cellerna för att minska friktionen när den växer, eller öka rotdiametern.

Det finns begränsat med tidigare kunskap om hur rottillväxt påverkas av dessa skillnader i tid och rum av olika tryck och syremängd i marken. Därför har syftet med detta arbete varit att försöka förstå hur två vanliga grödor som vete och ärt påverkas av variationer i syremängd, tryck men även en kombination av dessa två, i jorden.

Tillväxthastigheten och diametern på rötterna av ärt och vete mättes under sex dagar när plantorna växte i specialtillverkade lådor. Dessa lådor hade ett glas på ena sidan, därigenom kunde en kamera ta bild på rötterna var 20e minut under försöket. Utifrån dessa bilder beräknades sedan tillväxthastigheten. Efter försökets slut scannades rötterna i en flatbäddsscanner och utifrån dessa bilder mättes diametern i ett datorprogram. Efter att ha växt under optimala förhållanden i tre dagar utsattes rötterna för den första behandlingen. Vilken innefattade att rötterna utsattes för en syrefri miljö, att trycket ökade eller en kombination av dessa under 24 timmar. Dagen efter återställdes miljön till lägre tryck, syrerik jord eller både och. På den sjätte dagen utsattes rötterna återigen för de fysiska påfrestningarna i 24 timmar.

Vete och ärt påverkades lika av de tre olika behandlingarna, rötterna betedde sig likadant oberoende om de utsattes för syrebrist, ökat tryck i jorden eller en kombination av dem båda. Vete och ärt visade tydliga men olika mönster för tillväxthastighet mellan dagarna. Båda grödorna visade en minskning i tillväxthastighet av rötterna när de utsattes för syrebrist, högre tryck i jorden eller kombinationen. Skillnaden i hur vete och ärt reagerade var att ärt återhämtade sig i tillväxthastighet, den ökade igen, när jorden åter tillfördes syre eller trycket minskade på den femte dagen. Veteplantorna däremot visade ingen skillnad i tillväxthastighet på den femte dagen. De återhämtade sig alltså inte.

Diametern för ärtrötterna ökade under den första fysiska påfrestningen, men sedan under den andra påfrestningen på den sjätte dagen minskade dock diametern. För vete minskade diametern under alla tre sista dagarna. Dessa resultat för diametern är inkonsekventa och speglar inte mönstret för tillväxthastigheten som förväntat. I tidigare studier har en anpassning till dessa fysiska påfrestningar i jorden varit en ökad rotdiameter. Genom att istället mäta diametern direkt från bilderna tagna av kameran, och inte från en flatbäddsscanner, skulle resultaten kunna bli annorlunda.

Förmågan hos ärtrötter att återhämta sig i tillväxthastighet när jorden återigen blir syrerik eller trycket i jorden minskar, innebära att ärtrötter skulle kunna växa djupare ned i marken än veterötter efter att de stött på områden med olika tryck eller syremängd. Enligt dessa resultat kan ärt därför ha en mer positiv effekt på jordstrukturen och motverka försämrad jordstruktur djupare ned i marken än vete.

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1 Introduction

1.1 Importance of soils for agriculture and their spatial variability

Soils are important to produce food and clean fresh water, contribute to the diversity in the landscape and to energy and climate sustainability (McBratney *et al.*, 2014). There is increased pressure on soil resources as the world population increases. A consequence of this is a growing problem of degradation of soils globally (Koch *et al.*, 2013). The degradation of soil structure is often related to soil and crop management practices and land use (Bronick & Lal, 2005). Soil degradation includes processes such as organic matter decline, salinization, erosion, contamination, soil biodiversity loss, landslides, flooding and compaction (Jie *et al.*, 2002; McBratney *et al.*, 2014). Soil degradation has a negative impact on the quality and quantity of food production. Both crop yields and the concentration of micronutrients and protein in the plants declines, which negatively affects human health (Lal, 2009). Improving soil structure and decrease degradation is important for soil fertility and to increase future food production (Bronick & Lal, 2005).

The structure of the soil affects root and plant growth. Soil structure describes the spatial arrangement of liquid, solid and gas phases. Between those phases, most biological, chemical and physical processes and reactions in the soil occurs. The physical properties of a soil that control the growth of roots are water availability, aeration and resistance to root penetration (Angers & Caron, 1998). Too little water, hypoxic conditions or increased penetration resistance are major causes of limited root growth and plant development (Bengough *et al.*, 2011). These physical properties show strong spatial and temporal variability in the soil environment. The spatial variability of soil physical properties in fields is due to soil-forming factors but also by management practices like tillage (Iqbal *et al.*, 2005). Penetration resistance increases when the root enters a compact layer or aggregates and decreases when elongating through pores or cracks (Bengough & Young, 1993). The soil moisture differs with time as a result of varied precipitation (Baldrian, 2014) and due to the vegetation and the topography (Vereecken *et al.*, 2007). Also, the patchy distribution of hotspots with microbial activity crates localized zones of hypoxia (Borer *et al.*, 2018).

Soil provides physical support, nutrients, water and oxygen for plant roots (Doran *et al.*, 1996). At the same time, the plants positively affect the soil structure in different ways. One of the most important impacts in soil structure due to roots is the formation of continuous pores. In these pores, air and water can move and be stored and also decrease penetration resistance for future roots (Angers & Caron, 1998).

1.2 Threats to soils and their fertility

One soil degrading process that is increasing globally is compaction of soils. Compaction in field soils creates a combined physical stress of hypoxic conditions, drought and increased penetration resistance (Iijima *et al.*, 2007). The use of heavy machinery, intensive farming of crops and inappropriate soil management all contributes to compaction of the soil (Hamza & Anderson, 2005). One estimation is that 32 % of all European subsoils are compacted (Hargreaves *et al.*, 2019). In Sweden, compaction causes yield losses up to 15 % according to field experiments (Jordbruksverket, 2005). Pressure on the soil surface from machinery makes the soil particles more densely packed, soil porosity reduced and bulk density increased (Atwell, 1993). Air-filled pores are then less and smaller, and the diffusion of oxygen will be restricted in the soil. (Hargreaves *et al.*, 2019). As a consequence of this, the oxygen near the root surfaces is reduced (Patel & Mani, 2011). There is a compaction of both the topsoil and subsoil, but compaction of the subsoil is difficult to resolve and will last for a long time (Håkansson *et al.*, 1987).

Another increasing threat to soils and degrading process is the increase in flood events and local rainfalls due to climate change (Yamauchi *et al.*, 2018). Soils can become waterlogged due to poor drainage, and the risk increases if the soil is compacted (Drew, 1997; Bengough *et al.*, 2011). Globally 10-15 million hectares of wheat are estimated to be affected by waterlogging each year (Sayre *et al.*, 1994). Waterlogging is decreasing the gas diffusion of the soil (Sauter, 2013) and the gas exchange between the atmosphere and the soil. As a result of that, plant roots have to use the oxygen that is trapped in the soil (Shiono *et al.*, 2019).

1.3 Effects of physical stress on root and whole plant growth

In both compacted soils and hypoxic soils root growth is limited. Due to low root growth rate, less water and nutrients can be reached by the root system, which in turn has a negative impact on plant growth and eventually crop yield. Penetration resistance increases both in compacted and dry soils, then the force roots need to exert to penetrate the soil increases (Colombi *et al.*, 2018). In order for a root to elongate, the root tip must displace soil particles and overcome friction (Correa *et al.*, 2019). The expanding cells of the elongation zone create the force that pushes the root tip through the soil (Bengough *et al.*, 1997; Hamza & Anderson, 2005). The energy cost and required photosynthate increase with increasing penetration resistance (Atwell, 1990; Colombi *et al.*, 2019). The energy available for the shoot is then less which can lead to reduced plant growth and yield losses (Colombi *et al.*, 2019).

The risk of hypoxic conditions increases if the soil is compacted (Bengough *et al.*, 2011). During oxygen deficiency, the oxygen concentrations are rapidly reduced in the cells of the root (Bailey-Serres *et al.*, 2012), and the oxygen concentration declines with an increasing distance from the shoot. This is because oxygen is diffused from the shoot to the root tip. At the same time, oxygen is consumed by cells along the way and some is lost to the rhizosphere (Yamauchi *et al.*, 2018). After a few hours of heavy rain (Malik *et al.*, 2002) the oxygen concentration is on such a low level that the roots can suffer from oxygen deficiency which might limit the growth of the root (Kludze *et al.*, 1994; Yamauchi *et al.*, 2018).

How much a plant suffers from oxygen deficiency depends on the growth stage, the duration of the waterlogging and how deep the water levels are. Both yield and growth will be more affected if the plant faces hypoxia during an early vegetative stage (Malik *et al.*, 2002). de San Celedonio *et al.* (2014) speculated that a young plant can easier recover from hypoxic stress after the stress is released than if the hypoxic condition occurs later in the plant development. The growth of a young plant is also expected to be more affected than a mature plant if growing in compact soil, because then the whole root system will be in the compact part. A mature plant with a wide and deep root system might be less affected if only some parts of the root system is subjected to increased penetration resistance (Young *et al.*, 1997).

Root elongation is variable and sensitive to changes in the environment. The elongation rate can have large variations when the same species is growing under favorable conditions, like in fertile and loose soil. But if soil penetration increases or hypoxic conditions occur, the variation will be smaller (Pagès *et al.*, 2013). Different species is different sensitive against soil physical stresses. Plant species can withstand hypoxic conditions differently and most agriculture crops tolerate waterlogging poorly. Wheat is categorized as tolerant to oxygen deficiency and pea as sensitive (Vozary *et al.*, 2012). In compacted soils, according to Materechera *et al.* (1991), monocotyledonous plants like wheat are more sensitive to high strength soil than dicotyledonous plants like pea.

1.4 Adjustments of roots to soil physical stress

The root system is affected in different ways by soil physical stress, and roots may adjust to these changing conditions. When soil penetration resistance increases the root can increase the mucilage production and sloughing of outer root cap cells to decrease the frictional resistance (Iijima *et al.*, 2003). Another way to easier penetrate a high strength soil is to increase the root diameter. The increased diameter creates greater axial pressure and lowers the stress at the root tip. A thicker root is also less likely to buckle or bend when the root tip displaces soil particles (Materechera *et al.*, 1992; Chimungu *et al.*, 2015). The widening of the root is mainly due to an increased diameter of the cortical cells (Wilson, 1977), but can also be due to an increased amount of cells inside the root (Bystrova *et al.*, 2018; Colombi *et al.*, 2019). A morphological adjustment to hypoxic conditions in the soil is also an increase in root diameter (Blackwell & Wells, 1983). Thicker diameter contributes to an increased area where aerenchyma could develop and an increase in the oxygen flux (Jiménez *et al.*, 2015).

Another anatomical adjustment of oxygen deficiency is the formation of aerenchyma in the roots (Thomas *et al.*, 2005; Sauter, 2013; Yamauchi *et al.*, 2018). Aerenchyma is air-filled channels where gases can be transported rapidly with low resistance. They can be formed in roots by the death of the cortical cells or by the formation of gas spaces between cells (Yamauchi *et al.*, 2018). Plants can transport oxygen from the atmosphere through the shoot to the roots through aerenchyma to maintain aerobic respiration in the root. When suffering from oxygen deficiency, roots have to shift from aerobic respiration and use some anaerobic pathway like fermentation (Kludze *et al.*, 1994). Many plants use both lactic acid fermentation and ethanolic fermentation (Tadege, 1999). As a result of that, the energy cost of cell division and root growth increases (Herrmann & Colombi, 2019). For many plants like wheat, the formation of aerenchyma is induced by hypoxic conditions in the soil (Yamauchi *et al.*, 2018). In contrast, pea has no ability to develop aerenchyma when waterlogged, which makes pea more sensitive to hypoxic conditions (Ploschuk *et al.*, 2018).

A mechanism to tolerate hypoxic conditions is also adventitious formation. Adventitious roots can replace and support the primary root system (Sauter, 2013; Yamauchi *et al.*, 2018), and they can grow near the soil surface where the concentration of oxygen is higher (Shiono *et al.*, 2019). Due to the higher oxygen concentration in the upper layer of the soil, Haque *et al.* (2012) suggest that crops with shallow root systems could survive short-time waterlogging better. On the other hand, shallow root systems can reach less water and nutrients which are essential for plant growth.

1.5 Knowledge gaps and aims of this thesis

Efficient root systems that can tolerate physical stresses better is essential for crop development, to learn more about the effects of physical stresses on root growth is therefore important. Numerous previous studies have discussed the effects on root growth in high strength soils (Bengough *et al.*, 2011; Tracy *et al.*, 2011; Pfeifer *et al.*, 2014) and performed experiments on root elongation when oxygen is limited in the soil during hypoxic conditions (Kludze *et al.*, 1994; Yamauchi *et al.*, 2018). Also how pea or wheat roots respond to a combined stress of increased penetration resistance and waterlogging has been investigated by a few researchers (Saqib *et al.*, 2004; Iijima *et al.*, 2007). However, there is limited knowledge about the effects of fluctuations in soil physical properties on root development. A limited number of studies have investigated the recovery of roots after hypoxic treatment (Malik *et al.*, 2002; Araki *et al.*, 2012) and root recovery after growing in a high strength soil (Bengough & Young, 1993; Croser *et al.*, 2000). In these earlier studies, both the applied stress and the stress release lasted for several days, and the root growth and diameter were in majority measured after harvest. These relatively long time periods do not reflect the heterogeneity in soil, the physical environment a root is exposed to can change faster.

The aim of this study was to understand how pea (*Pisum sativum L.*) and wheat (*Triticum aestivum L.*) roots are affected in fluctuations of penetration resistance, the oxygen concentration and a combination of those in the soil. The following questions facilitate the achievement of this aim: i) How do root growth rate and diameter change when roots for 24 hours face higher penetration resistance, hypoxia or a combined stress? ii) do the growth and diameter recover after the first stress during the release? And finally, iii) how are root growth rate and diameter affected when the stresses are applied a second time during the growing period? In order to understand how roots respond to fluctuations in soil physical properties, the roots were grown in rhizoboxes in which soil physical conditions could be changed. A time-lapse imaging system was used to quantify the root growth rate, and the root diameter was measured from images.

2 Material and Methods

2.1 Substrate and plant material

Commercially available potting substrate (Ekojord, Hasselfors garden, Hasselfors, Sweden) was used in this study. This potting substrate was used due to its dark colour, good water holding capacity, nutrient richness and high porosity. The dark colour of the substrate created a clear contrast between soil and roots which is an advantage in image analysis. The soil was manually passed through a 4 mm sieve and stored in a plastic bag to avoid water loss. The bag was placed at 4 °C in darkness until use. The moisture of the soil was determined by drying the samples for 72 hours in an oven at 105 °C. The moisture was equivalent to 250 hPa suction as determined on a suction plate.

2.2 Design of customized rhizoboxes

The plants in this study were growing in rhizoboxes. Rhizoboxes are boxes filled with soil where the roots can be observed through a glass at the front side of the rhizoboxes (Moradi *et al.*, 2010). Rhizoboxes enables good control of the root environment, and measurements of roots at specific time intervals can easily be made and repeated (Nagel *et al.*, 2012). In the rhizobox the penetration resistance is equal over the entire soil volume, therefore the whole root system is exposed to the same degree of compaction.

The rhizoboxes were made of PLA plastic in a 3D printer (Ultimaker 3 Extended, Ultimaker, Geldermalsen, Netherlands). It consisted of five parts made in the 3D printer, a plexiglass, rubber tire, valves and air tubes. The inner dimension of the rhizobox are 12 cm x 4 cm x 1.4 cm (length x width x height). The different compartments of the rhizobox are illustrated in figure 1. The large hole on the top, when the rhizobox is in standing position, is where the plastic cone that contained the planted seedling was inserted. On the front side, a plexiglass was fixed with screws. In the back, a rubber tire was inserted which expands when pressurized air is pumped in through the valve on the backside. When the rubber tire is expanding the block on top of the tire is pushed forward to compact the growth substrate. When the cell is pressurized the inner dimensions are instead 12 cm x 4 cm x 1.1 cm. This creates a volume that is 14.4 cm^3 less than in the unpressurized state. Air tubes were connected on the bottom and top which enabled the flush of gas with different gas compositions through the whole soil volume.

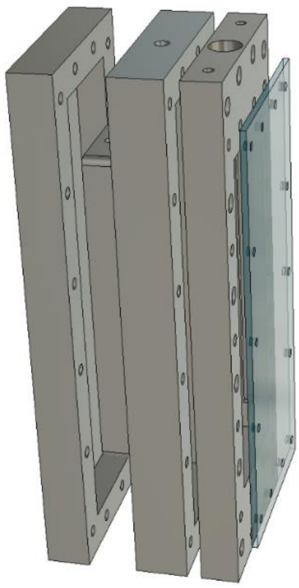

Figure 1. The different compartments of the rhizobox: a plexiglass in front, on top the hole where the planted seed is inserted, in the part on the back a rubber tire will be inserted with a smaller moving block in front.

2.3 Time-lapse imaging to record root growth

Twelve rhizoboxes were fixed in a metal frame constructed by aluminium bars inside a climate chamber. The set-up in the climate chamber is shown in figure 2. To favour the roots to grow near the glass, the rhizoboxes were placed at an inclination angle of 30° inside the chamber with the glass facing downwards. Behind the rhizoboxes a time-lapse imaging system was installed. A modified Canon EOS 750D (Canon, Tokyo, Japan) camera was used, in which the infrared filter was removed. The sensor had 24.2 megapixels resolution resulting in a pixel edge length of 20 µm. The settings of the camera to obtain optimal image quality consisted of an exposure time of 1/3 seconds, aperture value of f/13 and ISO of 100. A macro lens

(EF-S 35mm f/2.8 IS STM, Canon, Tokyo, Japan) was also used in combination with the camera. The distance from the camera lens to the rhizoboxes in the climate chamber was 17 cm. The camera was placed on a dolly at a conveyor belt. A stepper motor made the conveyor belt rotate and the dolly to move. The motor and the shutter were controlled by a programmed Arduino Mega from Arduino AG and the time was controlled by an Arduino Micro. The camera took pictures on the roots every 20 minutes and the images were saved as JPEG. To obtain the scale in the pictures, four size markers (labels with spots) were placed on the glass of the rhizoboxes. The time-lapse imaging system was placed in darkness and to be able to visualize the roots in the pictures, 20 infrared lights (λ=830 nm) (Vishay, Malvern, USA) were attached on both sides of the camera (Figure 2). Infrared light was used because it does not influence the growth of the roots.

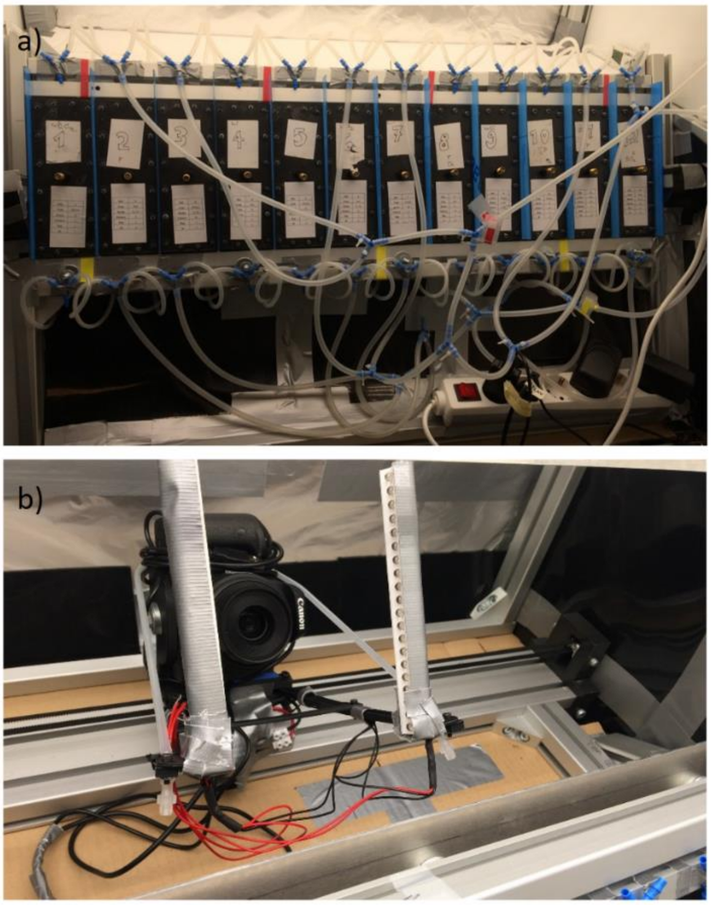

Figure 2. The experimental set-up: a) 12 rhizoboxes inside the climate chamber. The cells subjected to hypoxic conditions were connected to airtubes. b) The camera placed behind the rhizoboxes on a dolly at a conveyor belt, and infrared lights on the side of the camera lens.

2.4 Growth conditions and seed germination

The plants were grown for six days inside a climate chamber of model SED-41 (Percival, Perry, USA). In the climate chamber, the plants were exposed to 12 h light, air temperature of 19.2 °C and relative humidity of 58.3%. The rhizoboxes were filled homogeneously with the equivalent of 24 g dry potting substrate, which resulted in a bulk density of 0.36 g/cm^3.

Pea of the variety "Ingrid" and wheat of the variety "Rohan" were used in these experiments. Those crops were selected due to their importance in global food production. Pea is the third most important legume crop globally (Kaur *et al.*, 2012). Wheat is one of the most important crops for global food security (Shiferaw *et al.*, 2013) with a forecasted harvest in 2019/20 of 761 million tons ("International Grains Council," 2020). A dicotyledonous plant as pea and a monocotyledonous plant as wheat were also selected due to their differences in root systems. From the seed of a pea one primary root is growing. From the primary root, adventitious roots are later developed (Tricot *et al.*, 1997). From the embryo in the seed of wheat, embryonic roots emerge during seedling development. While adventitious roots later emerge from nodes (Yamauchi *et al.*, 2014). The number of primary roots varies, normally from one up to seven between different wheat plants and depending on the variety (Rich & Watt, 2013).

Pea and wheat seeds were pre-germinated in the dark between moist filter paper in petri dishes at 19.2 °C. Three days after the germination, seedlings with a primary root length of approximately 2 mm for wheat and 3 mm for pea were selected. The seedlings were planted in a plastic cone half-filled with soil, covered with a layer of loose soil. After they were planted 2 ml of water was added. The cone was covered with aluminium foil to prevent evaporation, but a small hole was left for the shoot to emerge unimpeded. To promote the root to grow near the glass a plastic root slide was inserted in the rhizobox that was pointing towards the glass (Figure 3).

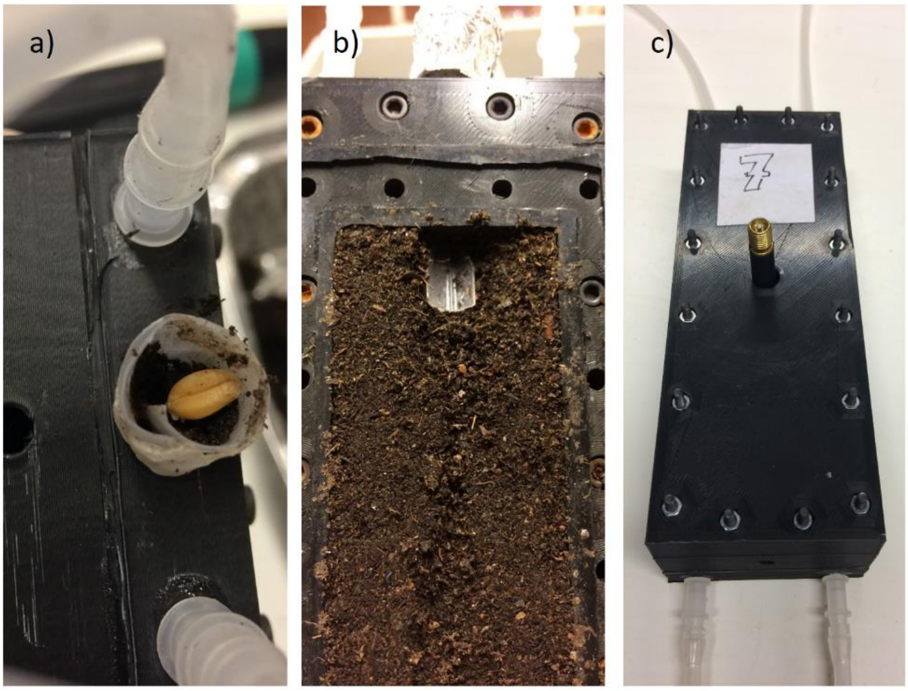

Figure 3. Planting and properties of the rhizobox: a) Planted seed in the plastic cone in the hole on top of the rhizobox. b) The plastic root slide. d) The valve on the back and airtubes from the bottom and top.

2.5 Treatments and control of stresses

Three treatments were applied on both wheat and pea. Those were hypoxic conditions, increased penetration resistance and the combination of both stresses. The plants grew for three days under optimal conditions. On the fourth day, the first physical stress was applied. The soil penetration resistance was increased by inflating the bicycle tire at the back of the growth compartment using compressed air (100 kPa air pressure). When pressurized, the bulk density in the rhizobox increased to 0.46 g/cm^3 which correspond to an increase of 0.1 g/cm^3 in comparison with the unpressurized state. Before the start of the experiment, all the rhizoboxes were pressurized and then unpressurized again to obtain equal starting conditions. In the hypoxic treatment, the rhizoboxes were flushed with nitrogen gas for a minute. During the remaining hours of the stress, the rhizoboxes were connected to a plastic bag (SKC Inc., Eighty Four, PA, USA). The bag, with a capacity of 25 liters, was filled with nitrogen gas and placed outside the climate chamber. The oxygen concentration in the rhizoboxes when nitrogen gas was added to the system was approximately 3 %.

During the release of the physical stress on the fifth day of growth, the pressure was released through the valve on the backside or the rhizobox was flushed with air. After re-aeration, the oxygen concentration in the rhizobox was around 21 %. The measurements of the oxygen concentration were performed in order to confirm there was no leakage between tubes and to confirm the flushing of nitrogen gas or air was successful. The measurements were made by the portable gas analyzer CheckPoint 3 (Dansensor, Ringsted, Denmark). On the sixth day of growth, the roots were subjected to a second physical stress for 24 hours before harvest.

In summary, soil physical stress was applied two times with a release of the stress in between, and all with a duration of 24 hours. There were also control plants that were not subjected to any soil physical stress. They were used to obtain root growth rate and the diameter under optimal growing conditions. These values could then be compared to the plants subjected to soil physical stress at the different time periods. All species-treatment combinations were replicated four times (n=4) and the different species-treatment combinations were randomly allocated in the climate chamber.

At harvest, the roots were cut off from the plant at the top of the rhizobox and gently removed from the soil. After that, the roots were cleaned from soil and preserved in tubes containing 70% ethanol and stored at 4 °C.

2.6 Computer-based root phenotyping from images

2.6.1 Root growth

The software ImageJ, version 1.52a (ImageJ, National Institutes of Health, Bethesda, USA) and R version 3.6.1 (R Core Team, 2019) were used to analyze the pictures taken by the camera. ImageJ could recognize the size markers in the pictures from the camera and their x- and y-coordinates. By clicking on the root tips in every picture, the coordinates of the root tips were obtained. In the software R, the coordinates of the size markers were transformed into millimeter and then the displacement of the rhizobox was calculated and corrected for in relative to the first image. Also the time and experiment duration for each picture was compiled in R. When knowing the scale, the coordinates for the root tips and the time, the root growth rate could be calculated. Within every time period (first stress, release and second stress) a mean value of the root growth rate was calculated for each root. For the wheat plants with more than one root, an average root growth rate was calculated of all visible roots. If the roots grew fast and reached the bottom of the rhizobox before the end of the experiment, the root growth rate was not calculated after they reached the bottom.

2.6.2 Diameter

After harvest, the roots were scanned in a flatbed scanner (Epson Perfection V800 Photo, Seiko Epson Corporation, Nagano, Japan) at a resolution of 1200 dpi and stored as TIF files. The diameters were measured in ImageJ from the pictures from the scanner. In ImageJ, the roots were divided into three parts. The first part contained the length the root elongated during the second stress, the second part the length the root grew during the release and the third part the length the root elongated during the first stress. After that, the diameter was measured at five random positions inside these three parts, avoiding the end of the root tip, where the root was bending or where a lateral was developing. A mean value was calculated for each time period for every plant, and for wheat plants with several roots, an average diameter was calculated. Same as for the root growth rate, if the roots reached the bottom of the rhizobox the diameter was only measured before it reached the bottom.

2.7 Statistical analysis

Statistical analyses were carried out in R. The following linear mixed model in combination with analysis of variance was used to test the effects of measurement time period, stress treatment, crop species and their interactions on root growth rate and root diameter. Linear mixed models were evaluated using the nlme package (Pinheiro *et al.*, 2019):

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta\gamma_{jk} + \delta_l + \varepsilon_{ijkl} \qquad (1)$$

Where Y represents the tested root trait (root growth rate, diameter) at the i^{th} measurement time period (i = stress 1, release, stress 2), in the j^{th} stress treatment (j = increased penetration resistance, soil hypoxia, combined stress), the k^{th} crop (k = pea, wheat) and the l^{th} sample (l = 1, 2, 3, …, 32). The effect of measurement time period (α), the stress treatment (β), and the crop (γ), as well as the interaction effects between measurement time period and stress treatment ($\alpha\beta$), measurement time period and crop ($\alpha\gamma$) and stress treatment and crop ($\beta\gamma$) were all treated as fixed effects. The effect of the sample (δ) was included as a random factor into the model to account for repeated measurements. The residual error is represented by ε. Mean values were compared using analysis of variance and least significant difference tests as implemented in the agricolae package (Mendiburu, 2019) at $p < 0.05$.

3 Results

3.1 Root growth quantification

Root growth rate was quantified from the pictures taken by the camera with the method described. The coordinates of the root tip was obtained every 20th minutes. The last image taken by the camera during the sixth day of growth, in comparison with the graph of root tip coordinates, is seen in figure 4.

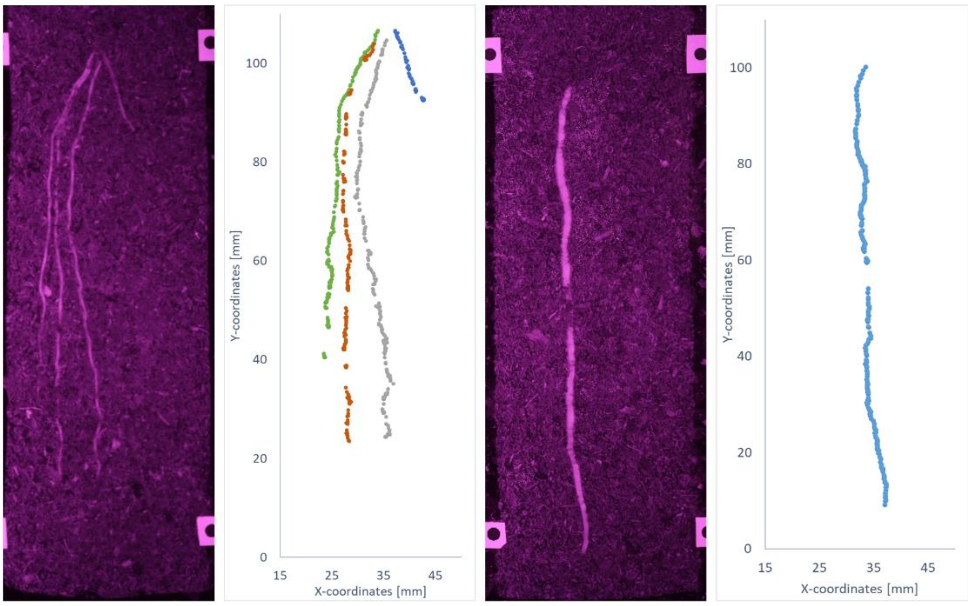

Figure 4. a) Typical image of wheat roots from a rhizobox at the end of the sixth growth day b) graph of the tip coordinates for the same wheat plant c) Typical image of a pea root d) graph of the tip coordinates of the pea.

3.2 Root growth rate and diameter in control plants

The control plants were not subjected to any soil physical stress. They were used to compare if there was a significant difference in diameter and root growth rate during the fourth, fifth and sixth days of growth. These days correspond to when the other plants were subjected to the first stress, the stress release and the second stress. Root growth rate and diameter did not differ significantly ($0.31 < p < 0.73$) between the three days (Figure 5). Therefore, one mean value of root growth rate and one value of root diameter was calculated for the entire time period.

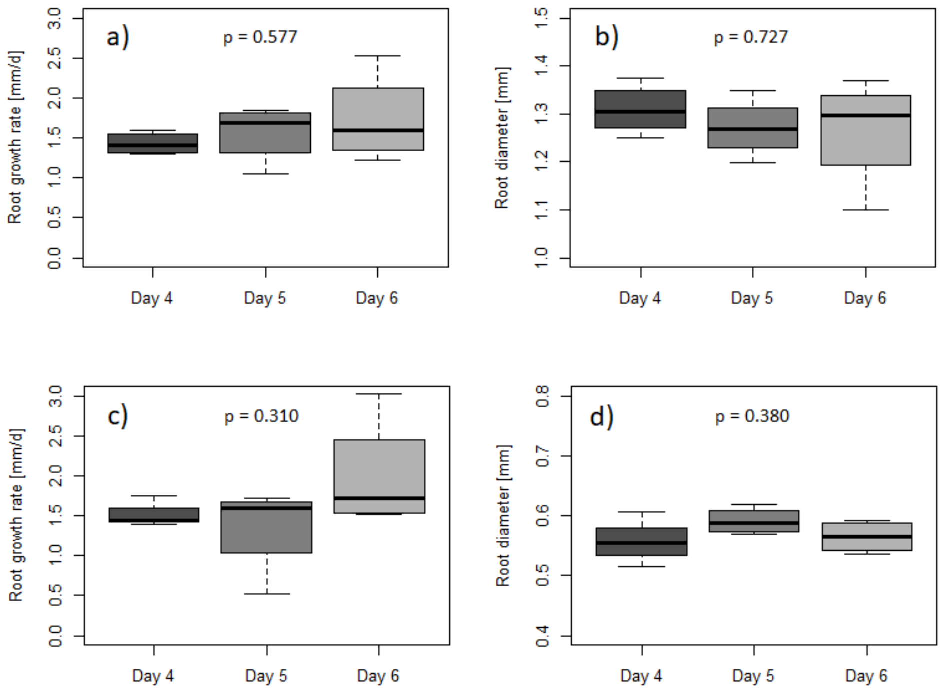

Figure 5. Boxplots showing the root growth rate (a, c) and diameter (b, d) in pea (a, b) wheat (c, d) at the 4th, 5th and 6th growth day under optimal growth conditions. P-values were obtained from analysis of variance.

3.3 Effects of soil physical stress on root growth rate

Results from the linear mixed model for the different effects of treatment, time period, crop and their interaction between those regarding root growth rate for pea and wheat are seen in table 1. The results showed there was no significant effect ($p=0.05$) of the treatments on root growth rate, which means that the roots responded similarly to the hypoxic, increased penetration and the combined treatment. There was a significant effect ($p < 0.05$) of the time period, indicating that root growth rate responded to changes in soil physical stress. A significant effect ($p < 0.05$) of the interaction between time period and crop was also observed. This shows that stress response patterns of root growth rate differed between wheat and pea.

Table 1. *Effects of treatment (increased penetration resistance, hypoxia and a combination), time (fourth, fifth and sixth day of growth), crop (pea and wheat) and their interactions on root growth rate for wheat and pea obtained from linear mixed models (Eq. 1; n=4).*

	Root growth rate [mm h^{-1}]	
Effects	**F-value**	**p-value**
Treatment	0.047	0.954
Time	6.085	0.005
Crop	0.019	0.891
Treatment:Time	0.214	0.929
Treatment:Crop	0.038	0.963
Time:Crop	6.955	0.003

In comparison with the control treatment, the root growth rate decreased during the first increase of soil penetration resistance or induced soil hypoxia on the fourth day of growth for both pea and wheat (Figure 6). The decrease in mean values compared to the control plants for pea was 28% for the increased penetration resistance treatment and approximately 21% for both the hypoxic and the combined treatment. For wheat, the reduction in root growth rate during the first increase of penetration resistance, occurring hypoxia or the combination was smaller, 16%, 17% and 15%, respectively. But for the release of physical stress, when the soil penetration resistance increased and hypoxic conditions occurred for the second time on the fifth day of growth, the crops responded differently. Regarding pea, all the treatments were significantly decreased between the fourth and the fifth day of growth, or significantly increased between the fifth and the sixth day of growth. This means the pea roots recovered during re-aeration or decreased penetration resistance on the fifth day of growth (Figure 6). During the sixth day of growth, when soil penetration resistance increased or soil hypoxia was induced for the second time, the root growth rate had a lower reduction compared to the control plants than for the hypoxic and increased penetration resistance treatment during the first stress on the fourth day of growth. The reduction in root growth rate during the second stress was then 16% for increased penetration resistance treatment and 19% for the hypoxic treatment compare to the control. For the combined treatment the reduction was instead 23% which is a larger decrease than during the first stress (Figure 6).

For wheat there was no significant difference in root growth rate between the fifth day, when there was a decrease of penetration resistance and re-aeration of the soil, compared to the day before when subjected to the first physical stress. This means wheat roots did not recover in root growth rate upon release of soil physical stress. On the sixth day of growth, when subjected to soil physical stress for the second time compared to the stress release the day before, there was a slight difference in root growth rate. In the increased penetration resistance treatment the reduction was 8%, a reduction of 13% in the hypoxic treatment and for the combined treatment there was instead an increase of 9% compared to the mean root growth rate during the stress release (Figure 6). However, these changes in root growth rate during the sixth day of growth were not significant different from the earlier time period.

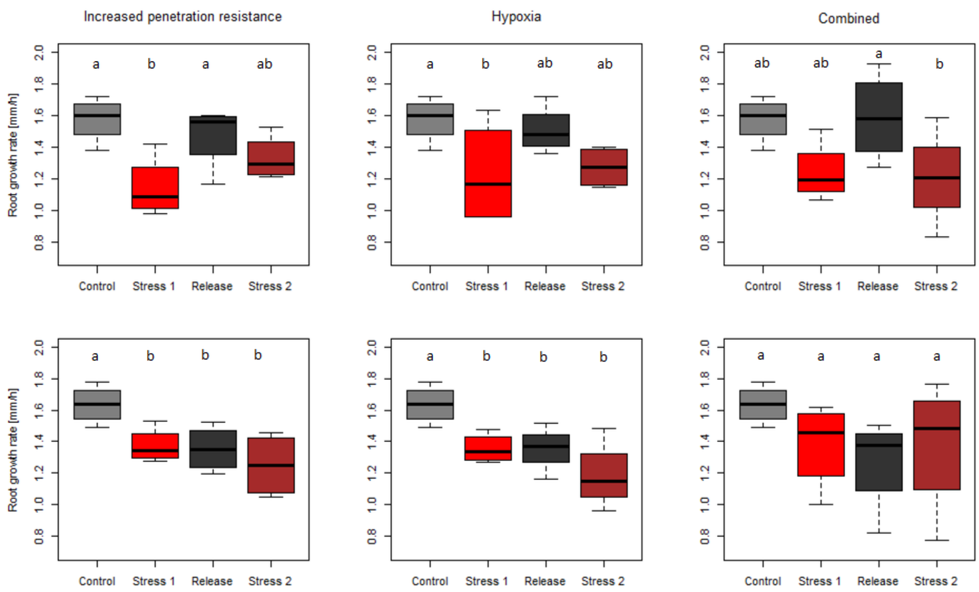

Figure 5. Boxplot showing the effects of soil physical stress on root growth rate for pea (upper row) and wheat (lower row). The boxplots illustrate the different treatments. Those to the left are for increased penetration resistance, in the middle hypoxic conditions and to the right the combination. Different letters indicate a significant difference in the mean using the least significant difference test ($p < 0.05$, n=4).

3.4 Effects of soil physical stress on root diameter

For root diameter of pea and wheat, the effects of treatment, time period, crop and interaction between those were obtained from the linear mixed model (Table 2). The results showed there was no significant effect (p=0.05) of the treatments on root diameter, indicating that roots response similar to the different physical stresses. There was a significant effect ($p < 0.05$) of the time period, which means the root diameter adjusted to fluctuations in physical properties over time for the treatments.

A significant effect ($p < 0.05$) on crop was also observed due to the larger diameter of pea roots compared to wheat roots. Regarding the interactions between time period and crop, there was a significant effect ($p < 0.05$) on the diameter, indicating that the pattern of responses to the stress differed between wheat and pea.

Table 2. *Effects of treatment (increased penetration resistance, hypoxia and a combination), time (fourth, fifth and sixth day of growth), crop (pea and wheat) and their interactions on root diameter for wheat and pea obtained from linear mixed models (Eq. 1; n=4).*

	Root diameter [mm]	
Effects	**F-value**	**p-value**
Treatment	0.550	0.586
Time	55.780	<0.001
Crop	766.008	<0.001
Treatment:Time	1.478	0.227
Treatment:Crop	0.908	0.421
Time:Crop	27.847	<0.001

In comparison to the control treatment, there was a significant increase in root diameter of pea on the fourth day of growth, when soil penetration resistance increased and when the roots were subjected to hypoxic conditions for the first time (Figure 7). The increase was 21% for the increased penetration resistance treatment, 14% for the hypoxic treatment and 18% for the combined treatment compared to the mean value of the control plants. After that, during the decrease of soil penetration resistance and when aeration was resumed on the fifth day of growth, the diameter decreased again. The decrease was then 9% for the increased penetration treatment, 10% for the hypoxic treatment and 15% for the combined treatment compared to the mean values during the first stress. When soil penetration resistance increased and when the roots were exposed to the hypoxic treatment again during the sixth day of growth, there was no significant difference or a slight decrease in comparison with the fifth day of stress release.

Regarding the diameter of wheat roots, there was a slight decrease or no change in diameter between the control plants and the first time soil penetration resistance increased or when soil hypoxia occurred on the fourth day of growth. Upon stress release on the fifth day of growth, there was a decrease in root diameter compared to the first day subjected to the physical stress for the increased penetration resistance (7%) and hypoxic treatment (5%). When the soil penetration resistance increased and when the roots were exposed to the hypoxic treatment for the second time during the sixth day of growth, there was no significant difference in comparison with the fifth day of stress release. Regarding the combined treatment there was no significant difference between any time period due to the large variation.

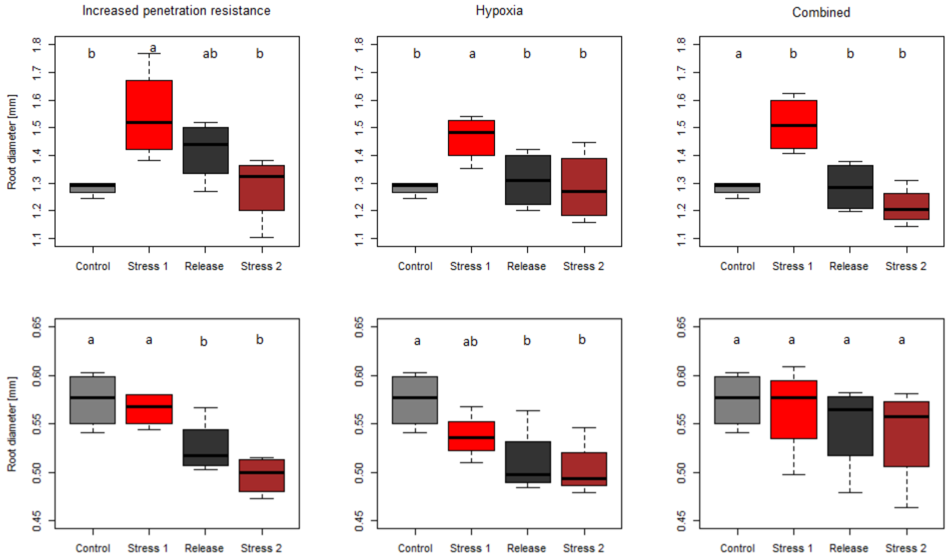

Figure 6. Boxplot showing the effects of soil physical stress on root diameter for pea (upper row) and wheat (lower row). The boxplots illustrate the different treatments. Those to the left are for increased penetration resistance, in the middle hypoxic conditions and to the right the combination. Different letters indicate a significant difference in the mean using the least significant difference test ($p < 0.05$, n=4).

4 Discussion

In this study, fluctuations in soil physical properties of penetration resistance, oxygen concentration and a combination of those were examined on root growth rate and root diameter. Two different crops were used, pea and wheat. The roots were subjected to the physical stress on the fourth and sixth day of growth, with a release of the stress on the fifth day between. The results indicated that pea and wheat respond differently in root growth rate and change in diameter when exposed to fluctuations in soil physical properties with time.

4.1 Responses of root growth rate to fluctuating soil physical stress differ between pea and wheat

Root growth rate decreased when subjected to soil hypoxia and increased penetration resistance in both pea and wheat (Figure 6). This reduction corresponds with previous studies, which reported that root growth rate is reduced under hypoxic conditions (Kludze *et al.*, 1994; Malik *et al.*, 2002; Yamauchi *et al.*, 2018) and due to increased soil penetration resistance (Materechera *et al.*, 1991; Tracy *et al.*, 2011; Pfeifer *et al.*, 2014). There was also a decrease in the combined treatment for pea, and during the first stress for wheat (Figure 6) which correspond with an earlier study of Iijima *et al.* (2007). In their study they measured a decrease in root growth rate when pea roots were subjected to a combination of increased penetration resistance and waterlogging for 48 hours (Iijima *et al.*, 2007). In our results, during the second stress for wheat there was instead an increase in root growth rate. However, the difference for wheat roots was not significant between the time periods. The no significant difference in root growth rate for wheat could be due to the large variation in the combined treatment.

During the re-aeration of the soil or decrease in penetration resistance on the fifth day of growth, pea roots recovered in growth rate while wheat did not (Figure 6).

Araki *et al.* (2012) and Malik *et al.* (2002) measured the root dry weight of wheat plants during hypoxic conditions and when aeration was resumed. The hypoxic stress lasted for several days and they measured a full recovery in root dry weight after growing for seven days in an aerated soil. This could be one reason why wheat did not recover during one day of re-aeration in our experiment, but we also had a shorter time period of stress. In Malik *et al.* (2015) study, pea was waterlogged for 14 days and after that the soil was aerated for 21 days. The root length recovered to the control value after the re-aeration (Malik *et al.*, 2015). This corresponds with the recovery in our experiment of pea root in the hypoxic treatment. The opposite of this, the recovery in growth rate of pea roots does not fully correspond with earlier studies where penetration resistance was increased. Bengough & Young (1993) were growing pea through a top layer of compacted soil for four days, and after that in a bottom layer of loose soil. The elongation rate was still reduced compared to the unimpeded elongation rate several days after growing out from the compact layer. In their study, the elongation rate did not immediately increase when penetration resistance decreased (Bengough & Young, 1993). Also, Croser *et al.* (2002) grew pea for three days in sand and after that moved the plants into hydroponics systems. The plants then continued to elongate more slowly than the control plants until 60 hours in the hydroponics system (Croser *et al.*, 2000). Presumably, in our study, the shorter time periods with one day of stress and release led to the differences in the results.

According to the results, when pea and wheat roots are subjected to increased penetration resistance or hypoxic conditions, the root growth rate decreases. This decrease corresponds to previous studies. However, during the re-aeration and decrease in penetration resistance, some earlier studies contradicted our results. The not corresponding results could be due to different length of the subjected physical stress and the release of the stress, or difference between varieties or environmental factors.

4.2 Inconsistent responses of root diameter to fluctuating soil physical stress

The diameter of pea and wheat roots were affected differently from physical stress. For wheat roots, the diameter decreased with time and did not recover. For pea roots, there was an increase during the fourth day of growth when subjected to the first physical stress, and after that a decrease in the fifth and sixth day of growth (Figure 7). According to previous studies, the root diameter increases in both pea and wheat when growing in high strength soils (Materechera *et al.*, 1991, 1992; Croser *et al.*,

2000) and that one adjustment to soil hypoxia is an increase in root diameter (Blackwell & Wells, 1983; Jiménez *et al.*, 2015). In our results, only the increase in diameter of pea roots during the first stress corresponds with the earlier studies. As the root growth rate was affected by physical stress, also adjustments to the stress like changing diameter were expected. Therefore, the pattern of the root diameter was expected to reflect the pattern of the root growth rate. An increase in diameter during the sixth day of growth when subjected to the second physical stress for pea roots and an increase in diameter during both the fourth and sixth day for wheat roots were also expected.

When the soil penetration resistance decreased again and during the re-aeration of the soil on the fifth day of growth, there was a decrease in root diameter of pea roots (Figure 7). Then the diameter recovered. A similar result was obtained in the study by Bengough & Young (1993) where roots grew for four days in a compact layer and after that for four more days in a loose layer of soil. There was no significant difference in root diameter when growing in the loose layer of soil in comparison with control plants. In Croser *et al.* (2000) study pea roots had the diameter measured after both 24 and 48 hours in a hydroponic system after growing for three days in sand. When growing in sand the root diameter was larger compared to the unimpeded roots. After 24 hours in the hydroponics, the increased penetration resistance still had an effect on the root diameter of the new root tissue and the diameter was still larger than the control values. But after 48 hours the diameter of the root tip was not significantly different from the unimpeded roots (Croser *et al.,* 2000). In Bengough & Young (1993) study the measurements on diameter were only made after growing for four days in the loose soil, but then the root diameter recovered to the same as the unimpeded control plants. Also the longer time period of 48 hours needed for the diameter of pea roots to recover in Croser *et al.* study compare to our results could be due to the longer time period subjected to the soil physical stress.

In our study, the roots did not consistently increase the diameter as a morphological adjustment to physical stress as in previous studies. Maybe longer time periods of stress are needed to measure a significant change in diameter. There could also be adjustments to the changes in physical stress on the cellular level inside the root that is only visible through microscopy. The inconsistent pattern of root diameter could also be due to the method used to measure the diameter. To scan the roots after harvest in a flatbed scanner and divide the roots into the three time periods of stress and stress release in the images was maybe not optimal. To measure the root diameter directly from the pictures taken by the camera, when the length of the root for each time period is easier to determine, could give different results.

In summary, a morphological adjustment to soil hypoxia or increased penetration resistance is an increase in diameter according to previous studies. However, the results showed that there was an effect of soil physical stress on root diameter but the response was inconsistent.

4.3 Implications for the crop tolerance to soil physical stress and soil structure dynamics

In field conditions, a combination of physical stresses normally affects the root system at the same time (Khan *et al.*, 2016). According to the results, a combined stress of hypoxic conditions and increased penetration resistance had similar responses and showed the same pattern as each stress alone. According to Grzesiak *et al.* (2014), a combination of several abiotic stresses can cause larger negative effects in contrast to a single stress. In their study seedlings were grown for four weeks in compacted soils, and during the last two weeks of growth the compacted soil was waterlogged. In their results, waterlogging caused a larger decrease in dry matter of roots in a severely compacted soil than in a soil with a low level of compaction (Grzesiak *et al.*, 2014). But in our study, there was no additive effect of the two physical stresses, the combined stress did not affect pea or wheat more than each stress alone.

Due to the spatial and temporal variability in soils (Iqbal *et al.*, 2005), crops will face fluctuations in soil physical properties during development from the seedling stage to the mature plant at harvest. In this study, the plant roots were subjected to soil physical stress two times during the growing period. Pea and wheat roots may continue to follow the same pattern in root growth rate as in the results when continuously subjected to fluctuations in penetration resistance or oxygen concentration in soil air during plant development. The root growth rate for wheat would then continue to decrease with time and would be considerably lower than the root growth rate for pea after subjected to physical stress multiple times. As a result of that, when subjected to soil physical stress several times during plant development, wheat roots will probably not elongate to the same depth in the soil profile as pea roots. Less water and nutrients can then be reached by the roots, which in turn affect plant growth and yield negatively. Pea roots will have a decrease in root growth rate each time it faces new physical stress. However, the root will recover in growth rate again upon stress release. Maybe the recovery of root growth rate will be smaller after each release of physical stress, but the root growth rate will not reach the same low root growth rate as wheat roots.

The formation of continuous pores by roots is important to improve soil structure (Angers & Caron, 1998). Pea, which recovered in root growth rate during re-aeration of the soil or when penetration resistance decreased again, can elongate deeper in the soil profile and create longer continuous pores than wheat when subjected to fluctuations in physical properties. Compaction of the subsoil is more difficult to resolve, especially by tillage, than compaction of the topsoil (Håkansson *et al.*, 1987). Roots are therefore of high importance to decrease the bulk density of the subsoil. Here, pea roots have a higher ability to improve soil structure in the subsoil and increase fertility than wheat roots. Roots of wheat plants will not elongate as deep as pea roots but probably form more adventitious roots that grow in the upper layer of the soil, this creates more pores that improve soil structure in the topsoil instead.

However, cultivation of fields destroys the continuity of the pores formed by roots, the pores are then cut off at plough depth. But the pores in the subsoil remains, they are only not connected to the surface anymore (Oades, 1993). The pores in the subsoil will as a result of this last in comparison with the pores created in the topsoil. Because of this, the results imply that pea roots are of greater importance for improving soil structure in agriculture fields where tillage is used. To gain the greatest benefits of roots in the sense of improving soil structure and to counteract soil degradation, the choice of crop which has the ability to elongate deep in the soil even though it faces fluctuations in physical properties, in combination with the type of soil management method must be considered.

4.4 Recommendations for future research

In order to understand the mechanisms underlying the results, root anatomy could be investigated through microscopy. The response of root diameter to physical stress was unexpected when comparing the results with previous studies. If the type or number of cells differed and if the cells were expanding or not between the time periods can be detected through microscopy. A change in the cellular level is possible even though it was not reflected in an increased diameter when the roots were subjected to physical stress.

Further investigations could also test to what extent these patterns of root growth rate and diameter hold for other varieties of wheat and pea. Because different varieties may respond differently to increased penetration resistance (Colombi & Walter, 2017) and to hypoxic conditions in the soil (Setter & Waters, 2003).

5 Conclusion

Pea and wheat showed different but consistent patterns regarding root growth rate during the time periods for the three treatments. Pea recovered in root growth rate upon re-aeration or decreased penetration resistance, while wheat did not. The pattern for the root diameter was inconsistent and did not reflect the pattern of root growth rate as expected. To measure the root diameter again from the pictures taken by the camera could make these results more consistent. In conclusion, different crops respond differently to fluctuations in soil physical properties, which in the long term affect their impact on improving soil structure. To counteract the degradation of agricultural soils, the choice of crop which can elongate deep in the soil even though it faces fluctuations in physical properties, is therefore of importance for future food production.

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