Doctoral Thesis

Elucidation of root-soil interactions of crops in space and time by establishment and application of novel image based non-invasive root phenotyping methods

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Elucidation of root-soil interactions of crops in space and time by establishment and application of novel image based non-invasive root phenotyping methods

A dissertation submitted to
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for the degree of
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presented by

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Summary

Root traits of crops are almost completely unutilized to improve the properties of cultivars in crop breeding to date. For this reason, it has been assumed that a great potential can be expected in the selection of cultivars with root systems that are better adapted and tailored for specific environmental target conditions. The selection process in crop breeding programs requires phenotypic data that can be correlated with genetic information. However, non-invasive phenotyping methodologies of shoot and particularly root growth processes are in large parts still under development. Recently, a remarkable progress in the field of root phenotyping has been achieved both in laboratory and field. However, widespread application of these modern phenotyping methods has only just begun and their value for crop breeding remains to be demonstrated.

The present thesis intended to contribute to the rapidly growing field of new methods in crop root phenotyping and monitoring by establishing novel in-depth soil-based phenotyping methods and evaluating the specific applicability of these methods for crop root phenotyping in soil.

Chapter 2 presents results from a rhizotron methodology that has been successfully established within this thesis. Rhizotrons are flat soil-filled growth containers with a transparent plate at which the roots grow along and therefore allow for imaging and analyses of root growth by appropriate software. The potential of rhizotrons could be shown when used in an automated phenotyping system located in the greenhouse. The robot system GROWSCREEN-Rhizo enables an automated and simultaneous imaging of root systems (root system architecture (RSA) and root growth dynamics) and shoots of various plant species grown in soil-filled rhizotrons. It has been shown that the roots visible at the transparent plates were representative for the total root systems and root biomass determined from washed root samples.

Root and shoot growth of barley under conditions of vertically heterogeneous soil compaction and heterogeneous nutrient availability was tested in split-root rhizotrons and the applicability of the rhizotron methodology was tested and applied to study compensatory growth effects (chapter 3). In vertically divided split-root rhizotrons single barley plants grew with one side of their root systems in one compartment and with the other side in the other compartment differing in soil compaction and fertilization. Roots in the loose compartment of the split-root treatment grew deeper when compared with uniform treatments. Moreover, compared with the uniform treatments, more lateral roots
were initiated in the compacted compartment in the split-root treatment, if only the compacted compartment was fertilized. Lateral root formation started a few days earlier in the loose compartments of the split-root treatments, irrespective whether the fertilization was performed only via the compacted compartment or via both compartments. These effects can be interpreted as compensatory growth adjustments to heterogeneous soil conditions and show phenotypic plasticity in barley RSA and root growth dynamics. In conclusion, it became evident that for phenotyping processes of barley root and shoot growth the first days after exposure to heterogeneous soil conditions are critical for the analysis of underlying physiological responses.

After establishment and test of the rhizotron methodology, the system was applied for the investigation of electrical impedance tomography (EIT) under controlled conditions in the climate chamber (chapter 4). Not only root density but also ion concentration, water content and dead root tissue in the soil had a strong effect on the polarization signal quantified by EIT. For this reason, it had been difficult to filter out the root signal from the mixed signal and it became evident that not so much the root density, but the ion concentration in the rhizosphere, had the strongest effect on the polarization signal. Consequently, results from chapter 4 showed that EIT is not an appropriate method for the direct characterization of root growth in soil within the near future.

In order to characterize more accurately the behavior of roots encountering biopores in soil, a method using X-ray computed tomography (CT) was developed for the laboratory scale (chapter 5). Cylinders were filled with silty loam from the field and three treatments differing with respect to soil structure were compared. ‘Trematropism’ – a tendency to grow towards a pore - could be observed clearly. It was shown that artificial pores improved root and shoot growth even if the roots did not grow predominantly in the pores. A lot of roots grew towards these pores, but when they reached them, only few roots grew into the pore and a large part of the roots reentered the bulk soil. For this reason, the pores helped to attract more roots towards the center of the compacted soil, when compared to the other treatments without artificial pores. Thereby a larger fraction of the root was brought in contact with the bulk soil and diminished the border effects of the pot wall. Thus, the observations presented here might be a beneficial indication how to set up controlled experiments in the future that are more closely approximating field growth situations. The results might even imply that also in the field, induced pores, either by mechanical loosening or by preceding crops, might ameliorate crop cultivation on compacted soils. Barley roots showed an impressively versatile behavior in the way
they explored the soil and the pores. These results supplied further indication for phenotypic plasticity with respect to shoot and particularly root growth in barley, which was already observed in the split-root study (chapter 3).

Several insights in root-soil interactions were achieved in this thesis. It could be shown that the used laboratory methods are valuable for future in-depth phenotyping, particularly for the study of soil physical parameters.
Zusammenfassung


Die vorliegende Arbeit zielte darauf ab, einen Beitrag auf dem rasant wachsenden Gebiet der Methodenentwicklung in der Nutzpflanzenwurzelphänotypisierung zu leisten. Neue bodenbasierte Phänotypisierungsmethoden wurden für detaillierte, spezifische Fragestellungen etabliert und für ihre jeweiligen Nutzungsmöglichkeiten im Bereich der Nutzpflanzenphänotypisierung in bodenbasierten Systemen bewertet.


GROWSCREEN-Rhizo ist ein Phänotypisierungs-Roboter, der eine vollautomatische und gleichzeitige Bildgebung von Sprossen und Wurzelsystemen (einschliesslich Wurzelsystemarchitektur und Wurzelwachstumsdynamik) von verschiedenen Pflanzenspezies, welche in bodengefüllten Rhizotronen kultiviert werden, ermöglicht. Im Rahmen dieser Arbeit konnte gezeigt werden, dass die an der Sichtscheibe gemessenen
Wurzelparameter repräsentativ für das Gesamtwurzelsystem und dessen Biomasse waren, welche an ausgewaschenen Wurzelsystemen bestimmt wurden.


Nach Etablierung und Test der Rhizotronmethode wurde das System zur Untersuchung der Elektrischen Impedanztomografie (EIT) unter kontrollierten Bedingungen in der Klimakammer verwendet (Kapitel 4). Es zeigte sich, dass nicht nur die Durchwurzelungsdichte, sondern auch die Ionenkonzentration, der Wassergehalt und totes Wurzelgewebe im Boden einen starken Einfluss auf das mittels EIT gemessene Polarisationssignal hatten. Aus diesen Gründen ist es schwierig, das Signal der Wurzel innerhalb des gemessenen Mischsignals eindeutig zu isolieren. Im Laufe der Untersuchung stellte sich heraus, dass weniger die Wurzeldichte selbst den stärksten X
Einfluss auf das Polarisationssignal hatte, sondern vielmehr die Ionenkonzentration in der Rhizosphäre. Folglich belegen die Ergebnisse des Kapitels 4, dass EIT zumindest in näherer Zukunft nicht als geeignete Methode für die direkte Charakterisierung von Wurzelwachstum im Boden betrachtet werden kann.


In der vorliegenden Arbeit wurden mehrere Erkenntnisse über Wurzel-Boden-Interaktionen gewonnen. Die verwendeten Labormethoden besitzen Bedeutung für die Anwendung im Rahmen detaillierter Pflanzenphänotypisierung und eigenen sich insbesondere für die Untersuchung von bodenphysikalischen Faktoren.
Chapter 1
General introduction

Insufficient nutrient and water uptake by roots has been identified globally as a major limitation to crop yields, particularly under conditions of low yield and less fertile (stress) environments (Lynch 2007, Tester and Langridge 2010, York et al. 2013). In future, crops will frequently be grown under conditions, which are less beneficial and suitable for crop production due to a lot of currently altering boundary conditions such as global climate change and shifting pressure of pests and diseases (Gregory et al. 2013). Effects of climate change are regularly adverse to crop production (Nelson et al. 2009). Moreover, crops must frequently be produced with lower inputs of fertilizer (Cordell and White 2011) and water for irrigation (Nelson et al. 2009, Herder et al. 2010) in the coming decades. It has been assumed that crop roots could have an extraordinary importance in the adjustment of crop management practices in this context, when their dynamic functions could be predicted and subsequently deployed more intensively than until today (Lynch 1995, Herder et al. 2010). Thus, a better knowledge about root processes could contribute to develop appropriate solutions to adapt cropping systems to future challenges. Moreover, roots may be important to increase the sustainability of crop production (Pierret et al. 2007).

The present thesis intended to contribute to the rapidly growing field of new methods in crop root phenotyping and monitoring by establishing and evaluating the specific applicability of selected novel methods for crop root phenotyping in soil.

1.1 Definitions of phenotype, phene and phenotyping

The phenotype is the ‘set of all types of traits of an organism or of one of its subsystems, however defined and construed’ (after Mahner and Kary 1997). Here it must be considered that the phenotype is determined by the interaction of genetics (gene expression, gene regulation and epigenetics) and environmental influences and therefore embraces the entirety of the observable physical, physiological and biochemical characteristics of an organism.
The term **phene** has been less well defined than the analogous term gene. Lynch and Brown (2012) characterized the phene as ‘any observable characteristic of an organism’. Thus, **plant phenotyping** has been defined as ‘the application of automated, high-throughput methods to characterize plant architecture and performance’ (Walter *et al.* 2012). A further definition of plant phenotyping is the ‘set of methodologies and protocols used to measure plant growth, architecture, and composition with a certain accuracy and precision at different scales of organization, from organs to canopies’ (Fiorani and Schurr 2013).

### 1.2 Breeding for crop root traits

The twenty-first century has been called the ‘century of crop breeding’ (Boller 2008, Stamp 2011), and crop root breeding in particular has been considered a priority for plant biology in this century (Lynch 2007, Lynch and Brown 2012). The use of phenotypic root traits in crop breeding has been almost completely unexplored to date (Khush 1995, Lynch 2007, Nagel *et al.* 2012, Silberbush 2013). Therefore, a huge potential can be expected here.

Roots have not been used to improve crop cultivars for two reasons. First, phenotyping root growth processes is highly challenging from a technical point of view (Furbank and Tester 2011). In particular, root phenotyping demands great effort and close collaboration between scientists from various disciplines (Furbank and Tester 2011, Fiorani and Schurr 2013, Dhont *et al.* 2013). In fact, first appropriate methods applicable for resolving root growth processes in the soil at a comparatively high temporal and spatial resolution have been developed only very recently (Silberbush 2013, Brumlop *et al.* 2013). Second, plant research did not focus on root-soil interactions until the 1980s. Until then, most studies had been conducted in nutrient solutions and were considered adequate by many researchers for understanding root functions and root-soil interactions such as nutrient uptake (Silberbush 2013). It has since become clear that root functions are much more complex and that hydroponic systems were not sufficient to understand specific root-soil interactions (Silberbush 2013).

Until now, crop breeding was mostly focused on the easily accessible traits of above ground organs, such as shoots, for example during the ‘green revolution’ (Borlaugh 2000). Breeding methods focused on shoot traits will likely stabilize and probably
increase yield in cereals (Khush 1995, Borlaugh 2000). Nevertheless, the yield increase in rice for example has slowed down in recent decades (Khush 1995, Peng et al. 1999). Simultaneously, the practical importance of the root system architecture (RSA) and root system development (over the growing season) for yield formation has been scarcely investigated (Khush 1995, Pierret et al. 2007, Silberbush 2013). In fact, for rice a superior grain filling percentage has been related to high root activity (Peng et al. 2008).

During the ‘green revolution’ crop breeding was conducted under conditions of high soil fertility (Lynch 2007). However, soil fertility may be a very precious resource in future. From a global perspective, insufficient nutrient and water uptake by roots have been identified as major limitations to crop yields, particularly under conditions of low yield and less fertile (stress) environments (Lynch 2007, Tester and Langridge 2010, York et al. 2013). Consequently, further progress in crop breeding can be assumed by deepening knowledge about root growth processes and root-soil interactions (Pierret et al. 2007, Lynch et al. 2007, Dodd et al. 2010, Nagel et al. 2012, Lynch and Brown 2012). A selection of root traits that improve water and nutrient uptake due to a tailored root system for specific agro-environmental conditions promises an enormous chance and potential to stabilize or even increase yields, particularly under less fertile soil conditions (Lynch 2007, Dhont et al. 2013). Shoot and root growth and development have to be quantified simultaneously in order to be able to understand their interaction and to derive the function of the whole system and resulting plant performance (Khush 1995, Peng et al. 2008, Noulas et al. 2010, Fiorani and Schurr 2013).

Besides the proposed potentials of selecting beneficial root traits in crop breeding programs, there are other good arguments for studying growth and development of root systems in soil. A better understanding of root-soil interactions would allow for an optimization of crop management practices. For example, better opportunities to predict how roots respond to different crop management practices and to changing environmental conditions could be useful to better prepare for these changes and to broaden options for controlling and influencing critical processes such as crop rotation and cultivar choice, soil cultivation (till or no till) and irrigation practices (Pierret et al. 2007, Dodd et al. 2010).
1.3 Crop root phenotyping

So far, plant phenotyping technologies lag behind genotyping technologies. This situation has been called the ‘phenotyping bottleneck’ (Furbank and Tester 2011). In particular root phenotyping is lagging behind.

The concept and the prerequisites for the task of breeding new root phenotypes that are tailored to enhance and optimize water and nutrient uptake have been called the ‘second green revolution’ (Lynch 2007). With respect to root phenotyping Lynch and Brown (2012) recommend to consider the significance of the recorded phenes for breeding and whether the phenes are useful. Lynch and Brown (2012) differentiate between unique and elementary phenes such as the root branching angle (unique and elementary phenes are frequently controlled by only a single gene), and phenes that are more general (e.g. quantitative traits) such as rooting depth and that are generated by several unique and elementary phenes. Rooting depth results from the root branching angle, but also from other phenes (see also York et al. 2013).

The following root traits (examples) have been considered useful and preferentially important in breeding programs:

• Root shallowness: shallow root systems have been shown to be beneficial for the exploitation of topsoil, which is frequently more fertile, particularly due to a higher mineralization and phosphorus content (Lynch 2007, Lynch 2013).

• Rooting depth: deep root systems reach deep soil water which is particularly beneficial under drought conditions (Gaiser et al. 2012).

• Root cortical aerenchyma: a better aeration of the root and a ‘cheaper’ construction with regard to consumption of assimilates to build up root tissue can be achieved by an appropriate aerenchyma (Lynch 2013).

• Gravitropism, geotropism: this phene is an important trait for the generation of deep root systems (Richards et al. 2010).

• Root branching angle: the branching angle affects shallowness, root length density and rooting depth (Lynch 2013).

• Root diameter: thick roots are related to an improved root penetration under conditions of high soil strength (e.g. Haling et al. 2011).
• Lateral root number, lateral root density, lateral root initiation: lateral roots can improve root density and are related to various soil conditions, such as soil compaction (Bingham and Bengough 2003).
• Number of whorls: importance has been shown for maize and bean. These phenes can affect root shallowness and root density (Lynch 2007, Lynch 2013).
• Root surface area and root length density: beneficial to nutrient uptake (particularly for mobile nutrients such as N), Noulas et al. (2010).
• Root hair length/density: related to nutrient uptake (particularly for immobile nutrients, such as P), Lynch and Brown (2012).

The aim of root phenotyping for crop breeding consists of an efficient achievement of relevant phenotypic information which is valid and reproducible on the field scale (for a review see Cobb et al. 2013, Araus and Cairns 2013). Results can be correlated with genetic information (such as sequencing-, gene expression- and QTL-data) for selection in breeding programs by marker-assisted selection (MAS) techniques (Tester and Langridge 2010, Walter et al. 2012). Genotyping methods have become very powerful (Byrne et al. 2013). In particular, genetic sequencing data can be gained comparatively fast, often highly automated and therefore convenient and cost-efficient.

1.4 Dynamic phenotypic data are required

Dynamic phenotypic data about root and shoot development (growth dynamics and architecture dynamics) are required because soil conditions are not stable in the field. Furthermore, the plants’ demand for water and nutrients is not constant throughout the growing period from germination to harvest. Moreover, it has been shown that growth processes and metabolite concentrations of plants are species-specific and frequently controlled by diurnal (diel) or circadian rhythms (Walter et al. 2009, Poiré et al. 2010, Ruts et al. 2012). Consequently, the time of day at which the measurement is performed may affect the result.
1.5 Why use *in-vitro* root phenotyping systems?

Typical *in vitro*-phenotyping systems use nutrient solution (or aeroponics), gels made by nutrient solution, such as agar-filled petri-dishes (Nagel *et al.* 2009) or gellan gum (Topp *et al.* 2013), or growth pouches (germination paper covered by plastic, Trachsel *et al.* 2009, Hund *et al.* 2009a) as growth medium. Compared to in-depth soil-based phenotyping methods, *in vitro*-phenotyping systems offer a high throughput at low cost. This is crucial for breeding progress (Lynch and Brown 2012). Furthermore, using growth pouches important environmental conditions such as nutrient availability can be controlled and changed within the experiment much more rapidly than in soil-based systems. Gregory *et al.* (2009) and Lynch and Brown (2012) proposed a concept to efficiently achieve valid phenotyping results. They suggested designing screening-sequences for phenotypic traits, starting with high-throughput (*in vitro*) systems, followed by in-depth phenotyping systems. For root breeding, *in vitro*-systems have to be considered as indispensable main component in the phenotyping sequence as specified in more detail below. However, it has been shown that it can be difficult to transfer results from laboratory (*in vitro*) methods to the field scale (Palta *et al.* 2011) because the achieved results are – depending on the investigated phenes – either not reproducible in the field or do not help to predict the field performance of root growth development and RSA in later growth stages (Watt 2012). For this reason, Gregory *et al.* (2009) and Lynch and Brown (2012) suggested that a practicable way for future phenotyping strategies may involve the combination of rather simple methods offering a high throughput (e.g. agar-gel based systems, growth pouches) and more in-depth examinations of root–soil interactions using laboratory methods such as rhizotrons (i.e. soil-based systems with a transparent plate which the roots grow along and are therefore partly visible at the plate), X-ray computed tomography, MRI/MRT, PET, neutron radiography (see definitions below) or field methods such as shovelomics (Trachsel *et al.* 2011), pinboard methods or profile wall methods (Böhm 1979), mini-rhizotrons (Faget *et al.* 2010), and maybe PCR-based methods (Mommer *et al.* 2010).
1.6 Why use soil-based root phenotyping systems?

Results from laboratory (*in vitro*) methods are often not reproducible in the field. There may be several reasons for this fact:

- In soils, the availability of water and nutrients is spatially and temporally inhomogeneous. It is difficult to mimic the spatiotemporal dynamics of limiting resource conditions of field soils in (artificial) phenotyping systems (Lynch and Brown 2012). Due to this challenge the plants’ growth response might be different in the laboratory and in the field.

- Biotic factors can be highly complex in the field soil. Thus, as roots interact with microorganisms in the soil, the plants’ response in the phenotyping system may not be as diverse as in the soil of the reference field. As an example, root-microbial interactions and symbioses (e.g. mycorrhiza) can have a large effect on nutrient uptake, particularly for phosphorus (Lambers *et al.* 2013). It is difficult to mimic the soil-microflora in the laboratory.

- The monitored growth period in the phenotyping system may be too short and therefore may not allow for a prediction of yield or root and shoot growth traits in later growth stages (Richards *et al.* 2010, Watt 2012).

From the difficulties of realistically mimicking the target field conditions, it becomes clear that on the one hand, the phenotyping systems have to be designed thoroughly and as realistically as possible (close to target field conditions), and interpreted cautiously. On the other hand, due to the economic need to keep costs low, the systems have to be designed simply. Several economic trade-offs have to be considered at the phenotyping scale (cost of measurement of the phene with available system; efficiency of the phene to achieve the desired breeding aim) and on the level of the plants’ metabolic balance (cost of the phene with respect to consumed assimilates used to build up root tissue; effects on the overall performance of the plant by the selection for this specific phene).

An accurate control of all specific environmental conditions relevant in the field (e.g. water content, nutrient content) is technically not always easily feasible in soil-based phenotyping systems to date. Consequently, these factors at least have to be thoroughly characterized and monitored (Poorter *et al.* 2012). However, the characterization of all
relevant physical and chemical soil conditions (water content and availability, oxygen content, temperature gradients, porosity and pore size distribution, soil mechanical resistance, ion concentration, mineralization) usually demands a lot of effort.

Recently, a couple of methods were developed that might drastically increase the knowledge gain in in-depth root phenotyping systems, since they allow for a better description of heterogeneous soil conditions. These methods can be combined with root phenotyping methods and will likely improve the understanding of the whole system (soil-plant-atmosphere continuum):

- **Planar optodes:** Blossfeld and Gansert (2012) have developed an advanced methodology for the monitoring of chemical and physical changes in the rhizosphere in soil-filled rhizotrons at a high spatial and temporal resolution by use of planar optodes. Planar optodes are 2D sensor foils that contain a layer of an analyte-specific fluorescent dye (fluorophore) that changes its emission properties depending on the concentration of a chemical substance (analyte) such as ammonium or hydroxide ions. Due to the fact that optodes are read out remotely by optical cameras, the optode foil can be inserted in growth containers such as rhizotrons below the transparent window plate. This way, the roots grow along the optode foil and the signal can be read out without using cables led through the transparent plate of the growth container. Thus, quantitative measurements can be achieved not disturbing the root-soil system by opening the growth container. The method allows for a non-invasive imaging of rhizosphere processes, which is a great advantage compared to conventional micro-electrodes. Because the sensor foils can have a size up to several centimeters, it is feasible to monitor huge regions of the rhizosphere.
- **Green fluorescent protein (GFP) and analogous proteins:** Faget *et al.* (2010) presented a method that allows for the discrimination of roots of single individuals or species in mixed crop stands in the field observed by mini-rhizotrons. Using this method, root-root interactions (e.g. inter-species competition) can be studied. This is an almost unexplored field of investigation. Faget *et al.* (2010) used individual plants that were genetically modified and expressed the green fluorescent protein (GFP). Thus, Faget *et al.* (2010) could identify the plants by exciting the GFP molecules in the roots with fluorescent light and measuring the signal in mini-rhizotrons by a mini-camera equipped with appropriate filters. Today, further proteins have been developed that emit light in various colors.
• X-ray computed tomography (CT): provides 3D views of objects by imaging via X-rays in multiple views, which are reconstructed to volumetric images. In case of questions regarding the characterization of structured soil and its plasticity (specifically in three dimensions), X-ray computed tomography has proven very valuable (Peth et al. 2013). Moreover, with respect to plants, it has been reported that X-ray computed tomography, when appropriate protocols are used, is most probably not harmful to growing plants since no difference to non-scanned controls could be observed (Flavel et al. 2012).


• Electrical resistivity tomography (ERT): is based on measuring the electrical resistivity between several configurations of electrodes coupled to the object under investigation. By means of tomographic inversion algorithms (see for a review Kemna 2000) 2D or 3D images of the electrical resistivity distribution within the object are reconstructed. ERT has proven useful for the characterization of water content in the field soil, which was correlated with water uptake by roots (Michot et al. 2003, al Hagrey 2007, Amato et al. 2008, Srayeddin and Doussan 2009). Furthermore, root mass distribution of tree roots was highly correlated with electrical conductivity in the soil-root system (Amato et al. 2008).

• Neutron radiography: this method is based on 2D imaging of flat samples (e.g. rhizotrons) via neutron beams. Neutron beam intensity and scattering after passing the sample is detected by a scintillator plate. Due to the fact that the method is very sensitive to hydrous substances, it can be used to image the water content in the rhizosphere of living, transpiring plants (Carminati et al. 2010).

• Positron emission tomography (PET): can be used to image metabolite transport dynamics in real-time. Plants are incubated with substances and molecules (such as $^{11}$CO$_2$) containing short-lived positron-emitting radiotracers ($^{11}$C, $^{13}$N). The radiation (annihilation gamma photons) outside the sample is detected in multiple views by scintillator plates and used for reconstructing 3D volumetric images (Kiser et al. 2008).

• Quantitative PCR-based methods: this approach can be utilized for the assessment of root mass density and the microbial community and related functional classes in soil (Mommer et al. 2010). Since specific genes (or fragments) and similar are detected, several specific RSA traits cannot be determined.
Optimally, root phenotyping would be conducted non-destructively and directly in the field (without the necessity for digging out the soil and the roots). However, a method that allows for a realistic, valid and non-invasive continuous measurement (monitoring) of root growth processes in the field does not exist to date. Glass plates or tubes inserted in the field soil (rhizotrons or mini-rhizotrons) in order to study root growth have led to advancements in root research because they saved labor, led to a lower disturbance of the soil-root system compared to huge dug-out holes and allowed for continuous measurements of roots at low costs (Bates 1937). However, mini-rhizotrons have some clear limitations: they are not applicable for quantification of RSA-traits due to limited observation space (Polomski and Kuhn 2002) and they are presumed not to representatively reflect natural root growth processes, due to artifacts generated at the glass-soil interface, as discussed by Smit et al. (2000).

Excavation of roots in the field, as performed by the shovelomics method in Trachsel et al. (2011) is valuable for characterizing final or interim RSA traits, but such methods do not allow for continuous measurements. Therefore, a particular challenge is the task of monitoring root growth processes (root system architecture parameters such as rooting depth, root density) over a period of time at an appropriate spatial resolution in natural soils in the field. This widely unsolved problem was one of the major aims of the present thesis and was approached by means of electrical impedance tomography (EIT).

Soil-based systems are indispensable to prove the reproducibility of root traits under realistic conditions and when effects of - and the plants’ responses to - specific environmental factors such as soil structure parameters (soil strength, soil compaction, soil pores/porosity) are intended to be studied. Soil compaction is known as the most serious environmental problem caused by agricultural activities such as soil cultivation by heavy and inappropriate machines and is one of the most substantial issues in crop production and land use (Tracy et al. 2011). Detrimental effects on the physical properties and structure of soil frequently occur due to soil compaction such as decreased macropore proportion, increased mechanical resistance, decreased hydraulic and gas conductivity. These negative effects on soil structure can facilitate irreversible damages such as erosion events (Hamza and Anderson 2005), hamper growth of roots and shoots and frequently result in persistent yield decline (Håkansson and Reeder 1994, Chamen et al. 2003). It is reasonable to study the effects of soil structure on root and shoot growth performance in soil-based phenotyping systems. Soil-based non-invasive phenotyping systems that allow for continuous measurement of root growth are difficult to establish and are therefore still
underrepresented, even in the laboratory. However, such systems are needed for basic (modeling) and applied research (breeding). For these reasons and requirements, the present thesis focused on non-invasive crop root phenotyping systems based on soil as a growth substrate. The application of modern phenotyping methods has only just begun and new techniques facilitate the investigation of numerous questions regarding root and shoot growth and development and the interplay of root-soil interactions that could not be investigated before. Possible applications of root traits targeted to improve both efficiency and sustainability of crop systems, comprise, besides the already named applications directly improving yield (water and nutrient uptake, also pest control), a considerable number of further tasks, which are indirectly and in the long term beneficial for agriculture, such as erosion control, soil amelioration (e.g. heavy metal uptake, improvement of soil structure) and groundwater protection for instance. Many relations between crop roots and environment go beyond yield and yield quality.

1.7 Crosslinks between global agricultural challenges and phenotyping

From a global perspective, agricultural cropping systems have to be prepared for - and adapted to - several challenging changes in future. Proceeding climate change will affect the conditions of agricultural production in different parts of the world with such factors as invasion of pests, diseases and weeds from other regions or increasing virulence of existing pests, changing season length, changes in the amount and distribution of precipitation, temperature of soil and air, and consequently the velocity of mineralization (Nelson et al. 2009). Therefore, climate change is involved in numerous aspects of human activities including agricultural practice and yield potential. Furthermore, climate change and agricultural processes are cross-linked via the global carbon balance, greenhouse gas emissions (animal husbandry and CO₂, nitrous oxide and methane emissions from the soil) and land use (e.g. draining, desertification, deforestation) (Moss et al. 2010).

Another set of problems is the increasing scarcity of non-renewable resources including oil, arable land and fertile soil (due to problems such as soil degradation, salinization and erosion), as well as clean water, particularly in developing countries. For example, soil fertility has been on the decline for decades already (Montgomery 2007). Megacities, where huge amounts of food are needed, expand particularly in regions with high soil
fertility (Peng et al. 1999, Kraas 2007, Koohafkan 2007). All these resources are crucial for agricultural crop production.

Approximately 842 million people are currently suffering from hunger and due to several causes this number is even at risk of increasing (UN World Food Program 2013). Amongst other reasons, this is also due to the hyperexponential growth of the world population. More food has to be produced within the next 50 years than was produced until today (Clark 2009). There is a consensus that it will be necessary to at least stabilize crop yields or where possible increase them, by breeding for cultivars with higher yield potentials (Khush 1995, Tester and Langridge 2010).

For increasing yield it is crucial to optimize and control all factors relevant for plant productivity. Proper root growth, root function and optimal root distribution in soil (extent and density, RSA) build essential factors and prerequisites in this context (for a review see Gregory et al. 2013). However, root growth, function and RSA can be negatively affected by many constraints such as biological factors (pests and diseases, Herder et al. 2010, Quentin et al. 2013), physical factors (mechanical strength, hydraulic conductivity, matric potential) and chemical factors (toxicity such as high aluminum concentration, salinity, acidity, low water content and low nutrient concentration). In contrast, some factors can be positively related to root functions such as mycorrhiza and appropriate soil structure (increasing soil aeration, mineralization and soil temperature for example). Furthermore, the functions of plant internal signaling (hormones) and gene expression on root growth processes and root morphological plasticity are not yet completely understood. Since the function of roots is not exploited in this respect until today, plant biological research increasingly focuses on root growth processes and soil-root interactions. For intensifying root research, appropriate, effective and versatile phenotyping methods for measuring root growth dynamics and root morphological changes are urgently required. A successful increase of the root system to explore soil resources is, along with photosynthesis, the basis of plant growth and, therefore, the basis for agriculture. For this reason, root improvement could be a key for a more sustainable agriculture and subsequently for local and global economy and food security.
1.8 Hypotheses

*General objectives*

The present thesis aimed on the investigation of a series of relevant hypotheses and questions regarding the interaction of crop root growth with soil. Particularly, effects of heterogeneous soil compaction, heterogeneous nutrient availability, and the spatial distribution of macropores on the exploration of the soil by the roots were studied. Furthermore, this thesis intended to contribute to the currently rapidly growing field of new methods in crop root phenotyping and monitoring by evaluating the specific applicability of the novel methods. The single chapters (2, 3, 5) present results from already established non-invasive root growth phenotyping methods (X-ray computed tomography (CT) and rhizotrons) and results from a new method (EIT, chapter 4) that had not been tested in root phenotyping.

*Specific questions in the individual chapters 2-5*

**Chapter 2** presents a novel phenotyping robot system using soil-filled rhizotrons under semi-controlled conditions in the greenhouse. Several species were tested in the system and the validity and representativeness of the growth processes visible at the transparent plates compared to total root system size in the soil was investigated. The aim of this study was to design and deploy a prototype for an automated and simultaneous monitoring of root system architecture in 2D and shoot growth for plants grown in rhizotrons.

**Questions:** a) Is the part of the root system visible at the transparent plate of the rhizotron representative of the total root system? b) Is the correlation between the visible and hidden part of the root system dependent on the root diameter of different species or on environmental conditions? c) As an example, how do barley and maize plants respond to different levels of soil compaction with regard to root growth dynamics and root system development?

**Chapter 3** reports on a study that deepened the investigation from chapter 2 on the effect of soil compaction on root and shoot growth. In chapter 3, the compensatory growth
responses and phenotypic plasticity of barley root and shoot growth affected by vertically heterogeneous soil compaction and fertilization were studied in split-root rhizotrons in the climate chamber for the first three weeks of growth.

Questions: a) Can root and shoot growth processes affected by soil compaction be mimicked in our system? b) Do plants show compensatory growth effects when affected by heterogeneous soil compaction? c) What is the effect of nutrients?

Chapter 4 elaborates on the applicability of electrical impedance tomography (EIT) to monitor and characterize root growth in soil. In order to study this question under controlled conditions, soil-filled rhizotrons were used in the climate chamber.

Questions: a) Can EIT be developed into a non-invasive tool for monitoring of root growth and function in soil and in the field? b) Which soil factors (water content, ion concentration) may prevent an accurate estimation of root density?

Chapter 5 presents results from an experiment conducted with X-ray computed tomography (CT) in which the effect of artificial macropores in compacted field soil on root and shoot growth was investigated.

Questions: a) Do barley roots grow into artificially generated macropores (diameter 1 mm) which are pricked into compacted field soil, even when the compacted layer is penetrable by the roots and the supply of water and nutrients is always sufficient? b) How do the roots react inside the pores? c) Do the roots stay inside the pores or do they leave the pores again? d) In case of roots growing inside the pores, does this situation also affect growth and development of the shoot? e) Do the roots show trematropic growth (i.e. ‘tendency to turn into direction of a hole’; here: to grow towards a pore)?
Chapter 2

GROWSCREEN-Rhizo is a novel phenotyping robot enabling simultaneous measurements of root and shoot growth for plants grown in soil-filled rhizotrons

2.1 Abstract

Root systems play an essential role in ensuring plant productivity. Experiments conducted in controlled environments and simulation models suggest that root geometry and responses of root architecture to environmental factors should be studied as a priority. However, compared with aboveground plant organs, roots are not easily accessible by noninvasive analyses and field research is still based almost completely on manual, destructive methods. Contributing to reducing the gap between laboratory and field experiments, we present a novel phenotyping system (GROWSCREEN-Rhizo), which is capable of automatically imaging roots and shoots of plants grown in soil-filled rhizotrons (up to a volume of ~18 L) with a throughput of 60 rhizotrons per hour. Analysis of plants grown in this setup is restricted to a certain plant size (up to a shoot height of 80 cm and root-system depth of 90 cm). We performed validation experiments using six different species and for barley and maize, we studied the effect of moderate soil compaction, which is a relevant factor in the field. First, we found that the portion of root systems that is visible through the rhizotrons’ transparent plate is representative of the total root system. The percentage of visible roots decreases with increasing average root diameter of the plant species studied and depends, to some extent, on environmental conditions. Second, we could measure relatively minor changes in root-system architecture induced by a moderate increase in soil compaction. Taken together, these findings demonstrate the good potential of this methodology to characterise root geometry and temporal growth responses with relatively high spatial accuracy and resolution for both monocotyledonous

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and dicotyledonous species. Our prototype will allow the design of high-throughput screening methodologies simulating environmental scenarios that are relevant in the field and will support breeding efforts towards improved resource use efficiency and stability of crop yields.

2.2 Introduction

Plant roots provide key functions encompassing anchorage to the substrate, absorption of water and nutrients, storage, hormone production for coordinated plant development and communication with biotic and abiotic environment. The overall geometry of root systems and the architectural changes in response to environmental challenges play an essential role in growth and development, as well as in determining plant performance, productivity and fitness (Lynch 1995; Hammer et al. 2009). However, because of difficulties in observing and quantifying roots in soil and consequently, interpreting data, dynamic changes in root-systems’ architecture are less characterised than those occurring in the phyllosphere (Herder et al. 2010). In the past, breeding for new varieties with higher yield has mainly focused on optimising shoot biomass accumulation, geometry and function (Gonzalez et al. 2009; Xing and Zhang 2010). Recent simulations suggest that the contribution of roots and root-system architecture to enhancing yield has been underestimated (Hammer et al. 2009). The modelling approach by Hammer et al. (2009) indicated that the continuous increase in yield of maize in the USA Corn Belt over the past 70 years was directly influenced by modifications in geometry and function of root-system architecture. Further, Manschadi et al. (2006) found that the angle at which seminal wheat roots grow affects whole root-system architecture and consequently, water extraction capacity from the soil and plant productivity under water deficit conditions. These examples highlight that a better understanding of root-system structure and function is critical to improve resource use efficiency of major crops, especially under unfavorable environmental scenarios. These include not only water scarceness, but also low soil fertility and increasing salinity as well as erosion and soil degradation. Non-invasive, high-throughput phenotyping methods of root systems are indispensable for identifying genotypes with specific root system architecture resulting in increased ability to adapt plant development to changing environmental conditions. Novel technologies are required to characterise the complexity of root systems automatically to assist in
identifying heritable root traits. Selection for specific traits based on integration of molecular–mechanistic knowledge with accurate measurements of plant performance could be even more productive in breeding processes than conventional field screening (Lynch 2007; Passioura 2010).

Owing to practical reasons, phenotyping of root-system architecture under field conditions is challenging and still relies on traditional methods, e.g. manual measurements or visual estimations (De Smet et al. 2012). Roots have to be harvested destructively by labour-intensive excavation processes. Remarkably, however, dedicated and trained teams can visually score root traits of excavated adult maize plants within a few minutes (Trachsel et al. 2011). Non-destructive measurements of roots at frequent time intervals in the field are practicable by using mini-rhizotron tubes inserted in the soil (Gregory 1979; Johnson et al. 2001). However, the analysis of whole-root systems is not feasible because only roots growing along the transparent tube are accessible to cameras. Additionally, the variety and complexity of field situations can significantly impact root-system architecture (Lynch 1995; Clark et al. 2011) and makes the elucidation of the genetic and developmental basis of root-system architecture particularly challenging.

Combinations of field-, greenhouse and laboratory-based approaches are needed to address these questions. In laboratory conditions, plants can be subjected to controlled combinations of various abiotic and biotic stress factors simultaneously simulating environmental scenarios to which plants are exposed under natural conditions. Such approaches facilitate the identification of genetic components responsible for certain phenotypes or yield increases that may play as well a key role under field conditions. Typically, insights into root systems can be extrapolated from plants grown in artificial substrates, including transparent agarose gel or gellan gum (Nagel et al. 2006; Iyer-Pascuzzi et al. 2010), paper rolls (Zhu and Lynch 2004), growth pouches consisting of blotting paper covered by plastic foil (Hund et al. 2009a) and hydroponic cultures (Jones 1982; Tuberosa et al. 2002). These cultivation procedures combined with appropriate imaging setups allow optical visualisation and quantification of entire root-system architecture in 2D (Walter et al. 2002; Armengaud et al. 2009; Hargreaves et al. 2009; Nagel et al. 2009) or reconstructions in 3D if images from numerous camera view angles are acquired (Iyer-Pascuzzi et al. 2010; Clark et al. 2011). However, these methodologies have several drawbacks, such as absence of microbial interactions, soil structure and in most cases, even absence of mechanical impedance. In addition, it remains difficult to create heterogeneity of water and nutrient availability typically observed along soil
profiles (Hutchings and John 2004). To address these limitations, several laboratories have experimented with techniques to obtain information about root structure and function from plants grown in natural substrates, such as transparent soil-filled columns or rhizotrons (Thaler and Pagès 1995; Giuliani et al. 2005; Watt et al. 2006). The observation of roots at transparent interfaces is one of the earliest non-destructive techniques for studying root growth in soil and was first introduced in the 19th century (Sachs 1873). Shape and volumes of rhizotrons vary depending on the research objective and range from small boxes designed to study Arabidopsis roots in the laboratory (Devienne-Barret et al. 2006) to large containers, underground cellars, or walkways enclosing natural soil profiles under field conditions for direct observations of tree roots (Hilton et al. 1969; Taylor et al. 1990). The indisputable advantage of rhizotrons is the opportunity to perform repeated measurements of the same roots at frequent time intervals. When the thickness of rhizotrons is limited to less than 10mm and a translucent substrate is used, 2D light transmission images can be used to explore the dynamics of root water uptake of the root system (Garrigues et al. 2006). For opaque substrates, recently developed techniques like X-ray computed tomography (CT; Heeraman et al. 1997; Gregory et al. 2003; Pierret et al. 2003; Hargreaves et al. 2009; Tracy et al. 2010; Moradi et al. 2011) and nuclear magnetic resonance imaging (MRI; Menzel et al. 2007; Jahnke et al. 2009; Nagel et al. 2009) have made considerable progress. Both techniques facilitate the non-destructive investigations of 3D geometry of root systems grown in soil, but are not yet appropriate to phenotype root systems at a high-throughput rate (e.g. hundreds of plants per day). Additionally, frequent measurements of the same root system using CT should be avoided because of the risk of unpredictable effects of high energy radiation on plant growth. In summary, CT and MRI play an essential role in elucidating the mechanistic understanding of root structure and function, but for screening relatively large plant populations at high frequency and high throughput traditional optical sensors are more appropriate using scanner- or camera based image acquisition systems. Robotised equipment for imaging plants greatly facilitates high-throughput phenotyping, maximising speed and permitting standardisation. For screening shoots of monocot or dicot plants several techniques have been implemented (Granier et al. 2006; Jansen et al. 2009; Rajendran et al. 2009); however, automated systems for phenotyping root system architecture of plants grown in transparent soil-filled containers are lacking so far.

To start addressing these needs, the aim of this study was to design and deploy a prototype for automatically analysing root system architecture in 2D for plants grown in
rhizotrons. The novel setup, GROWSCREEN-Rhizo, allows simultaneous imaging of root and shoot growth of 60 rhizotrons per hour (total capacity of the setup are 72 rhizotrons). For validation two dicot (Arabidopsis and rapeseed) and four monocot (Brachypodium, barley, rice and maize) plant species were analysed with this setup and the hypothesis was tested whether the part of the root system visible at the transparent face of the rhizotrons is representative of the total root system. Further, we investigated whether the correlation between the visible and hidden part of the root systems is dependent on the root diameter of different species or on environmental conditions. In addition, we show the potential of the novel system by investigating the reaction of root growth dynamic and root system development of barley and maize plants to different levels of soil compaction.

2.3 Materials and methods

Plant material, experiments and soil cultivation protocols

To validate the novel system, we compared the vertical distribution of monocotyledonous and dicotyledonous root systems within rhizotrons (Experiment 1) and quantified the projected shoot area of monocotyledonous plants by analyzing images taken from different camera angles (Experiment 2). In addition, we tested the correlation of visible root length with total root-system length and plant development (Experiment 3) and the potential of the system was shown by analysing the effect of soil compaction on shoot and root growth and root-system architecture (Experiment 4).

In Experiment 1, the following plant species were analysed: Arabidopsis thaliana (L. Heynh.) ecotype Col-0, Brachypodium distachyon (L.) P. Beauv. (GRA 788, Genebank Gatersleben, Germany), Brassica napus (L.) cv. Campino (rapeseed) and Hordeum vulgare (L.) cv. Barke (barley). In Experiment 3 the same plant species were examined and additional Oryza sativa (L.) cv. Dom Sufid (rice, IRGC 117265, International Rice Research Institute, Metro Manila, Philippines) and Zea mays (L.) cv. Badischer Gelber (maize). Seeds of Arabidopsis, Brachypodium, rapeseed and rice were sown in small rhizotrons (60302 cm) and barley and maize were grown in larger rhizotrons (90603.4 cm). The rhizotrons, consisting of black or light grey polyethylene and one transparent polycarbonate plate, were filled with black peat soil (Graberde; Plantaflor Humus, Vechta
Germany; containing N, ~120 mg L\(^{-1}\); P\(_2\)O\(_5\), ~20 mg L\(^{-1}\); K\(_2\)O, ~170 mg L\(^{-1}\)). For correlation of projected leaf area with shoot biomass (Experiment 2) Zea mays (L.) cv. Helix and H. vulgare (L.) cv. Barke were cultivated in peat soil ‘ED73’ (Einheitserde, Balster Einheitserdewerk, Fröndenberg, Germany; N, ~250 mg L\(^{-1}\), P\(_2\)O\(_5\), ~300 mg L\(^{-1}\), K\(_2\)O, ~400 mg L\(^{-1}\)). In addition, to test the effect of soil compaction on root growth (Experiment 4), Zea mays (L.) cv. Badischer Gelber was sown in silty clay loam soil collected from a field site at the Klein-Altendorf agricultural station (University of Bonn) in Germany. Seeds of H. vulgare cv. Golden promise were germinated on filter paper and were transplanted when they were 4 days old into rhizotrons with different compacted black peat (Reiner Hochmoortorf; Florabella Tuintrof, Geeste, Germany; N, ~35 mg L\(^{-1}\); P\(_2\)O\(_5\), ~30 mg L\(^{-1}\); K\(_2\)O, ~40 mg L\(^{-1}\)) mixed with basalt grit (1.0 : 2.3 w/w). Fine powdered gardening lime (95% CaCO\(_3\), trace elements) was mixed with the peat (1 : 50) to adjust the pH of the substrate to 6.5.

To standardise compaction protocols across replicate rhizotrons, portions of 500 or 1000 g substrate were poured gradually and compressed as described below. The substrate was compacted using a custom-built compaction frame including a manual pallet fork-lift for lifting individual rhizotrons while applying a defined pressure to the soil surface by means of a wooden plank. Applied pressure and compaction values were calculated using a scale. Two draining drills with a diameter of 0.8 cm at the bottom of the rhizotrons, together with a layer of hygroscopic foam (10 cm, Mosy GmbH, Thedinghausen, Germany) maintained sufficient drainage and oxygen supply to the roots (~20% by volume, data not shown).

All plants were supplied with tap water (~7 mg L\(^{-1}\) N, 0.5 mg L\(^{-1}\) P, 2.6 mg L\(^{-1}\) K, 14 mg L\(^{-1}\) Mg; 440 mS cm\(^{-1}\)), except for rice and barley plants grown in the peat/basalt grid mix, which were supplied with nutrient solution (rice: 7.1 mmol L\(^{-1}\) N, 0.52 mmol L\(^{-1}\) P\(_2\)O\(_5\), 2.05 mmol L\(^{-1}\) K\(_2\)O, 320 mmol L\(^{-1}\) Mg, 7.45 mmol L\(^{-1}\) Si, 1.1 mmol L\(^{-1}\) Fe and barley: 24.9 mmol L\(^{-1}\) N, 1.3 mmol L\(^{-1}\) P, 1.75 mmol L\(^{-1}\) K, 27.9 nmol L\(^{-1}\) Si). To keep a soil water content of ~30% (VWC), plants were watered regularly and the frequency and amount of water or nutrient solution depended on the size of the rhizotrons (small rhizotrons: three times per week 60 mL; large rhizotrons: twice a day 400 mL). Plants were grown in the PhyTec greenhouse of the Institute Plant Sciences (IBG-2; Forschungszentrum Jülich GmbH, Jülich, Germany), which is covered by a specially formulated micro-structured glass (Centrosolar Glas, Fürth, Germany) with high transparency for photosynthetically active radiation (PAR) and ultraviolet (UV) radiation.
(up to 97% in visible light and up to 35% UV-B transmittance). Environmental conditions were: day length of 16 h, day/night temperatures of ~24/18°C and supplemental illumination (SON-T AGRO 400, Philips, Amsterdam, The Netherlands) was automatically turned on when the ambient light intensity outside the greenhouse was <400 mmol m\(^{-2}\) s\(^{-1}\) between 0600 and 2200 hours local time.

*Automated phenotyping of root-system architecture and shoot growth*

We designed the GROWSCREEN-Rhizo setup (Fig. 1) in collaboration with the company Maschinenbau Kitz GmbH (Troisdorf, Germany) who built the prototype and provided automation control. The rhizotrons were custom-built at Forschungszentrum Jülich GmbH and the final automation protocols and imaging setup were realised at our institute. The imaging platform enables measuring simultaneously development of leaf area and root systems for plants grown in up to 60 rhizotrons per hour. Plants can be analysed with this setup until shoot reaches a maximum height of 80 cm or roots reach the bottom of the rhizotrons (maximum depth of 90 cm). Consequently, the duration of experiments is restricted to a certain time period after germination corresponding, for example, to 4 weeks for maize plants or up to flowering time point for *Arabidopsis* plants in our conditions.
The prototype is located in the PhyTec greenhouse facility and consists of two rows of mounting frames in which rhizotrons (outer dimensions: 90 x 70 x 5 cm) are inserted. However, individual or multiple smaller rhizotrons can be inserted by using adapters. The rhizotrons consist of one transparent polycarbonate plate. To prevent light from reaching roots and also algal growth in the soil, the transparent side of the rhizotrons is shielded by an opaque plate combined with dense, black brush curtains. The inclination angle of the rhizotrons can be adjusted from 0° (vertical) to 43° with the transparent plate of the rhizotrons facing downwards. Rhizotrons are placed in two rows; each row is split into two groups which can be treated separately. The whole procedure is automated.

**Fig. 1.** GROWSCREEN-Rhizo, mechanical setup with 72 positions for rhizotrons that are aligned in two rows in the greenhouse. The inclination angle of the rhizotrons is adjusted to 43° with transparent plate of the rhizotrons facing downwards. The rhizotrons are split into four groups that can be treated separately. The insert picture (top left) shows the irrigation system exemplary of one rhizotron with four drippers (a) to ensure a homogeneous distribution of water or nutrient solution over the rhizotron. To prevent light from reaching roots and also algal growth in the soil, the transparent side of the rhizotrons is shielded by an opaque plate (b) combined with dense, black brush curtains (c; insert picture top right). Between both rows of rhizotrons a cabinet (d) is moved automatically on a linear axis with bi-directional motion (indicated by white dashed arrow) to the positions of the rhizotrons. In a user defined order, the rhizotrons are drawn inside the cabinet for image acquisition of roots and shoots. The whole procedure is automated.
automatically on a linear axis with a bidirectional motion. Users can define in which order the cabinet will reach rhizotrons for analysis. To draw a rhizotron into the imaging cabinet, the analysis sleds carrying cameras and light panels inside the cabinet are adapted to the angle of the compartment from which the rhizotron is being drawn. This ensures that rhizotrons are kept at the same angle during both cultivation and imaging. A change of the inclination angle would lead to a modified gravitropic signal. After adjusting the angle, the rhizotron is positioned inside the imaging cabinet by a mechanical swivel arm pulling each rhizotron at a hook mounted on one side. The motion into the cabinet is facilitated by slide bars and roller bearings. The motor drawing the rhizotrons is able to actuate completely sand-filled rhizotrons (up to 80 kg). Subsequently, the doors of the cabinet are closed with rolling cutter gates to prevent light conditions influencing image acquisition. Inside the cabinet, two side-view images of the shoot were acquired by two cameras (5 MP camera, GRAS-50S5C, Point Grey Research Inc., Vancouver, Canada; combined with 8mmFL compact fixed focal length lens, NT56–526, Edmund Optics GmbH, Karlsruhe, Germany) mounted at an angle of 90° to each other and one image of the whole transparent rhizotron surface is acquired with a high resolution camera (16 MP camera, IPX-16M3-VMFB, Imperx, Inc., Boca Raton, FL, USA; combined with Zeiss Distagon T 2,0/28 ZF-I lens, Jena, Germany). The resolution of the acquired images (230 µm per pixel) is high enough to detect the roots of the evaluated plant species. Illumination is provided by using LED-panels (LED Light Source SL3500-W-J, cool white, colour temperature 8000 K, Brno, Czech Republic), which are turned on in sync with image acquisition. This temporary illumination pattern, equal to all plants, did not produce any significant effect on root growth that could be revealed by comparing undisturbed and regularly screened plants (data not shown). The position and angle of the light panel were adjusted to prevent reflections in the images. To increase the contrast between plant and background and to avoid reflections, the cabinet is equipped with black walls. After image acquisition the gates are opened and the rhizotron is placed back to its initial position completing the routine. These steps are repeated automatically for each user-defined position. The whole procedure is automated and driven by a custom software program implemented with LabVIEW (National Instruments, Austin, TX, USA).

For automatic irrigation of plants, a system (T1030plus, Gardena Deutschland GmbH, Ulm, Germany) was installed equipped with four drippers per rhizotron (Fig. 1). The drippers are uniformly distributed over the length of the rhizotrons and allow irrigation of the plants at a user-defined frequency and volume (± 2%). Each rhizotron contains two
drainage holes to release gravimetrically the excess irrigation solution, which is released into a canalisation system mounted below the rhizotrons and can be collected for physical–chemical analyses. Sensors can be installed inside the rhizotrons to monitor, for example, soil moisture content, soil temperature, or pH and oxygen with planar optodes (Blossfeld et al. 2011) respectively.

Analysis of root-system architecture

Images and image sequences of root systems acquired with GROWSCREEN-Rhizo were analysed using the software GROWSCREEN-Root as described, with modifications (Mühlich et al. 2008; Nagel et al. 2009). We originally developed this software to quantify root growth and root system architecture of plants grown in agar-filled Petri dishes. With agar-grown plants, whole-root systems are visible and automatic tracking and extraction of root traits can be done routinely (Nagel et al. 2009) as only the portion of the root system growing along the transparent plate of rhizotrons is accessible to imaging (Fig. 2). Some roots grow temporarily or permanently within the soil substrate. Consequently, it is not possible to extract a complete tree model for the whole-root systems of rhizotron-grown plants, which is the requirement of the software GROWSCREEN-Root (for details see Mühlich et al. 2008; Nagel et al. 2009). As a result, we adapted the software to allow manual tracking of those roots that could not be detected automatically. Manually tracing roots can be time consuming. Using computer mouse graphics tablet with pens (Wacom Cintiq 21UX, CANCOM Deutschland GmbH, Düsseldorf, Germany) to trace individual roots can increase the speed of image analysis. Additionally, we implemented a batch analysis routine to overlay root structures of subsequent images for any given time series. This feature further reduces time for analysing images by tracing only newly developed roots. The time required for image analysis depends on the complexity of root systems and the frequency of image acquisition and varies between minutes to hours. We conclude that, to reach the goal of matching the same throughput in image acquisition and processing especially for complex root systems and low contrast backgrounds the software will need to be further improved in the future. The structure of all roots – manually or automatically detected – is then integrated, depicted in a false-colour image (Fig. 2b, d) and used to determine the following root parameters: root length, branching rates and angles and spatial distribution of roots within the substrate. Root traits can be divided into global ones – those derived
from the entire visible part of the root system; and local ones – those derived from individual roots. Global traits include total length of all visible roots, root length density (root length per surface area of rhizotrons) quantified at certain substrate layers, rooting depth representing the maximal vertical depth of a root system and root system width representing the maximal horizontal width of a root system. Traits resulting from performance of individual roots comprise length and number of roots including different root orders, such as main roots (including shoot borne roots) and lateral roots (Fig. 2) branched from main roots as well as angles of roots. Branching angles of lateral roots represent the angle between a main and a branched lateral root and emerging angles of main roots represent the angle between horizontal and main roots. The novel device GROWSCREEN-Rhizo enables the measurement of the same individuals repeatedly in a userdefined frequency (hours or days respectively). Consequently, all root traits can be quantified at a single time point or related to dynamic changes in characteristics of root-system architecture.

To correlate visible roots (from 2D imaging) with total root length and biomass, roots were carefully washed out of the soil and scanned (600 dpi, flatbed scanner, Canon Scan LIDE 60, Canon, Krefeld, Germany). Total root-system length was then determined either by tracing roots with GROWSCREEN-Root or with a commercial software (WinRHIZO 2012, Regent Instruments; settings: grey value threshold 30; removal of objects with an area <1 cm$^2$ and a length-width-ratio <4). Dry weight of both roots and shoots were determined after samples had been oven-dried at 70°C for ~48 h or until constant weight was reached.
Fig. 2. Representative original and colour-coded images with main roots (in green) and lateral roots (in red) of an Arabidopsis (a, b) and Hordeum vulgare cv. Barke (c, d) plants grown in soil-filled rhizotrons. The higher resolution image (e) shows an area of interest – indicated in (c) – with x5 magnification.
Analysis of shoot growth and estimation of shoot biomass

For monocotyledonous plants like maize and barley, colour images from two side-views at a 90° horizontal rotation were used to quantify the projected leaf area. The number of pixels corresponding to projected leaf area was determined automatically with custom-made algorithms that allowed segmentation for thresholds of the parameters hue, saturation and value and therefore distinguishing between plant and background (Walter et al. 2007). To compare the projected leaf area quantified from images with real leaf area, leaves of each maize and barley plant were scanned (300 dpi, flatbed scanner, Canon Scan LIDE 60, Canon). For these purposes, plants were harvested at different developmental stages up to 6 weeks after sowing. At each time point, 10 maize and barley plants were harvested and fresh weight of shoot was measured to correlate shoot biomass with detected leaf area.

Statistical analysis

The effect of mechanical impedance on root growth and spatial distribution of roots within rhizotrons were analysed using Student’s t-test (SigmaStat, Systat Software Inc., Richmond, CA, USA).

2.4 Results

GROWSCREEN-Rhizo enables quantification of root and shoot growth non-invasively

To evaluate the precision of the software tool for analysing growth and geometry of visible parts of root systems growing along the transparent plate of rhizotrons, reference objects with defined lengths were inserted in rhizotrons. The strong linear correlation ($R^2 = 0.999$) between the real length and the length of those objects quantified with the software GROWSCREEN-Root point out the high precision of the novel image-based tool and its value for root phenotyping (Fig. 3). Based on this, we could, for instance, analyse the vertical distribution within rhizotrons of both monocotyledonous and dicotyledonous root systems (Fig. 4, Experiment 1). Generally, dicots exhibited a higher root length density in the upper than in the deeper soil layers. The dicot model plant...
Arabidopsis exhibited a root length density of up to 0.9 cm cm\(^{-2}\) surface area of rhizotrons in the top 15 cm, which strongly decreased in deeper substrate layers (Fig. 4a). In rapeseed, a similar result was found with a root length density of up to 0.8 cm cm\(^{-2}\) in the upper 15 cm of rhizotrons (Fig. 4b). Nevertheless, at a comparable root-system length of \(\sim\)260 cm, root system of rapeseed plants reached deeper substrate layers compared with Arabidopsis (55 vs 30 cm respectively). Consequently, root length density of rapeseed plants declined less sharply in deeper zones of the rhizotrons.

In contrast to dicots, Brachypodium and barley produced fewer roots in the upper soil layers. Both plants exhibited the maximal root length density already in the top 5 cm, however, with lower average values: 0.7 cm cm\(^{-2}\) (Brachypodium) and 0.5 cm cm\(^{-2}\) (barley) respectively. On the basis of this contrasting behaviour in the top soil together with a more gradual decrease of root length density in deeper substrate layers of monocot compared with dicot species, the spatial distribution of monocots and dicots varied significantly \((P < 0.05\) at depth of 10–13 cm and 26–36 cm (model species; Fig. 4a); \(P < 0.05\) at depth of 6–13 cm and 33–46 cm (crop species; Fig. 4b) respectively). These
observations indicate that this method facilitates the quantitative evaluation of the spatial
distribution of roots within the soil profile, which represents a valuable root trait
connected to water and nutrient accessibility.

To calculate projected leaf area during shoot development of monocotyledons we used
images taken from two side-views at a 90° horizontal rotation angle. To evaluate the
precision of the analysis, the image-based method was calibrated against destructive
measurements of total leaf area and shoot biomass (Experiment 2). When the sum of
projected leaf area of both 2D images was compared with leaf area quantified by scanning
leaves, we found that linear regression captures the variation (Fig. 5a, b). The leaf area
determined from the sum of projected leaf area from both images seem to slightly
overestimate total leaf area of maize (~2%) and even more for barley plants (~12%) due
to more complex shoot architecture of the latter. Despite this overestimation the
correlation coefficient for 100 barley plants at different developmental stages (up to 6
weeks after sowing) was $R^2 = 0.97$ (Fig. 5a) and for 80 maize plants even larger $R^2 = 0.99$
(Fig. 5b). Similar linear correlations were found when the projected leaf area estimated
from the two side-view images was plotted against the shoot biomass ($R^2 = 0.95$ for
barley and $R^2 = 0.98$ for maize plants, Fig. 5c, d). This result implies that leaf area
quantified non-invasively by images taken from two side-views at a 90° horizontal
rotation can be sufficient to estimate shoot development at early vegetative stages.

Fig. 4. Spatial distribution of roots visible at the transparent surface of soil-filled rhizotrons analysed
with GROWSCREEN-Root. Root length density distribution of two model species, Arabidopsis and
Brachypodium (a) and two crop species, Brassica napus (rapeseed) and H. vulgare cv. Barke (barley,
b) was compared at equal root-system length (~260 cm). Plants were grown at a soil compaction level
of ~0.07 MPa and rhizotrons were set to an inclination angle of 43° (mean value ± s.e., n = 4–5).
The visible portion of the root system in rhizotrons is correlated with the total length of the root system for different species.

Our novel screening device was specifically designed to enable standardised routine evaluation of growth and architecture of roots grown in soil-filled rhizotrons non-invasively. However, one disadvantage of rhizotrons is that only a part of the root system is visible at the transparent plate of the containers. The proportion of roots reaching the transparent plate that is accessible for image analysis is dependent on the inclination of the rhizotrons with respect to the ground (Experiment 3). Generally, the more the rhizotron is inclined (with the transparent side of rhizotrons facing downwards), the higher the proportion of visible roots compared with the entire root system. Although only ~14% of the total root system of barley plants grown in vertical rhizotrons...
(inclination angle of 0°, representing the angle between the vertical line and the rhizotrons) was visible, this percentage increased to ~24% at an inclination angle of 25° and was ~33% at an inclination angle of 43° (representing the maximum inclination angle of the GROWSCREEN-Rhizo setup) respectively (data not shown). In additional to the inclination angle of rhizotrons, we tested if soil properties, in particular mechanical impedance affect the fraction of visible roots. Although a moderate increase in soil compaction by 2–3 times (up to 0.16 MPa, maize and 0.78 MPa, barley) compared with low compacted soil resulted in specific root weight increases for both barley (+38%) and maize plants (+11%), the fraction of visible roots was only marginally reduced for barley plants (~2%) and slightly increased for maize plants (+4%, Table 1). In contrast to the inclination angle of rhizotrons, we observed that moderate soil mechanical impedance on developing roots had a negligible effect on the proportion of roots which are visible at the transparent plate of rhizotrons.

Table 1. Effect of soil compaction and correlation of visible root length at the transparent surface of rhizotrons with total root length (extracted from fitted linear regression curves for each plant species and growth condition; correlation coefficients (R²) are given) and specific root weight (mean value ± s.e., n = 5–21).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Soil compaction level (MPa)</th>
<th>Ratio visible vs total root length (%)</th>
<th>Root biomass per root length (mg m⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hordeum vulgare</em> cv.</td>
<td>0.30</td>
<td>29.4% (R² = 0.87)</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td><em>Golden promise</em></td>
<td>0.78</td>
<td>27.2% (R² = 0.72)</td>
<td>6.5 ± 0.6</td>
</tr>
<tr>
<td><em>Zea mays</em> cv.</td>
<td>0.07</td>
<td>16.7% (R² = 0.51)</td>
<td>24.5 ± 5.1</td>
</tr>
<tr>
<td><em>Badischer Gelber</em></td>
<td>0.16</td>
<td>20.3% (R² = 0.94)</td>
<td>27.4 ± 9.6</td>
</tr>
</tbody>
</table>

For further confirmation of the correlation between the visible and total root-system length, we analysed four monocot and two dicot plant species under comparable growth conditions including inclination angle of rhizotrons of 43° (for more details, see ‘Material and methods’). Linear correlations were found between the root length visible at the transparent surface of soil-filled rhizotrons and the total root-system length for all examined plant species (Fig. 6a). The correlation coefficients ranged from R² = 0.91 for barley plants up to R² = 0.97 for rapeseed plants, with the exception of maize (R² = 0.51). However, the slopes of linear regression curves varied between species: both examined
dicot species (*Arabidopsis* and rapeseed) showed curves with steeper gradient compared with the monocot species, rice, barley, *Brachypodium* and maize respectively (Fig. 6a). These results show that the percentage of visible roots compared with total root system differs between plant species in our setup. *Arabidopsis* roots grown in rhizotrons positioned on average 77% of the entire root system along the transparent plate and rapeseed plants ~42%. In the examined monocot species comparatively less roots are visible; 33% barley, 32% rice, 24% *Brachypodium* and only 17% of maize root system are accessible (Fig. 6a; Table 2). To some extent, the fraction of roots visible along the transparent plate was related to the specific root weight for the examined plant species. The higher the proportion of visible roots, the lower the specific root weight, which ranged from 0.5 mg m\(^{-1}\) in *Arabidopsis* to 24.5 mg m\(^{-1}\) root biomass per unit root length in maize plants (Table 2). One exception was *Brachypodium*, which exhibited a relatively low fraction of visible roots together with a low specific root weight of 1.7 mg m\(^{-1}\).

Further, we tested to what extent the visible root length may also be a measure for root biomass. Similar to the correlation of visible root length with total root length, we found that the visible fraction correlated with root dry weight of different plant species (Fig. 6b). Furthermore, visible root length exhibited linear correlations with development of aboveground plant organs, shoot biomass (Fig. 6c) as well as leaf area development (Fig. 6d). Comparable to the results obtained for the correlation between visible and total root-system length, the slopes of linear regression curves differed between plant species. *Arabidopsis* plants exhibited the steepest gradient compared with rapeseed, rice, barley and *Brachypodium* plants; maize showed the weakest gradient (Fig. 6). Accordingly, at a comparable visible root length of 300 cm, maize plants produced 75 times more root biomass, 14 times more shoot biomass and a leaf area 9-times larger than *Arabidopsis* plants.
Fig. 6. Correlation between root length visible at the transparent surface of soil-filled rhizotrons with total root-system length (a), root (b) and shoot (c) biomass as well as leaf area (d) of Arabidopsis (n = 14), Brachypodium (n = 14), Brassica napus (rapeseed, n = 19), Hordeum vulgare cv. Barke (barley, n = 23), Oryza sativa (rice, n = 30) and Zea mays (maize, n = 21) plants grown in rhizotrons. Plants were grown at a soil compaction level of ~0.07 MPa and rhizotrons were set to an inclination angle of 43°.
Table 2. Correlation between visible root length at the transparent surface of rhizotrons with total root length (extracted from fitted linear regression curves of each plant species; correlation coefficients ($R^2$) are given) and comparison of specific root weight of different plant species (mean value ± s.e., $n = 11–30$). Plants were grown under a soil compaction level of ~0.07 MPa and rhizotrons were set to an inclination angle of 43°.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Ratio visible vs total root length (%)</th>
<th>Root biomass per root length (mg m$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabidopsis</td>
<td>77% ($R^2 = 0.96$)</td>
<td>0.5 ± 0.05</td>
</tr>
<tr>
<td>Brassica napus</td>
<td>42% ($R^2 = 0.97$)</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>Hordeum vulgare cv. Barke</td>
<td>33% ($R^2 = 0.91$)</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>Oryza sativa</td>
<td>32% ($R^2 = 0.94$)</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>Brachypodium distachyon</td>
<td>24% ($R^2 = 0.95$)</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>Zea mays cv. Badischer Gelber</td>
<td>17% ($R^2 = 0.51$)</td>
<td>24.5 ± 5.1</td>
</tr>
</tbody>
</table>

Moderate increases in soil strength affect root-system architecture of barley plants

As a first application of the novel system GROWSCREEN-Rhizo, we devised a protocol to study the reaction of root growth dynamic and root-system development in response to varying soil compaction levels in rhizotrons (Fig. 7, Experiment 4). Soil compaction is a factor that may significantly limit the development of root systems in the field. To understand the potential of the system, we chose to apply a relatively moderate soil compaction level of 0.52 MPa (moderate compaction) compared with low compaction of 0.06 MPa (low compaction). The outcome of this relatively small increase in soil strength was a comparable leaf area development (Fig. 7a) with similar shoot growth rates ($14.4 \pm 1.3\%$ day$^{-1}$ for low, vs $15.5 \pm 1.2\%$ day$^{-1}$ for moderate) as well as similar leaf mass per area values ($22.9 \pm 0.6$ g m$^{-2}$ for low, vs $21.9 \pm 1.3$ g m$^{-2}$ for moderate) of barley plants grown under both soil compaction levels. In contrast to the shoot, root systems of barley plants responded significantly to these small changes in compaction levels. The increased soil compaction led to 26% shorter main root length compared with plants grown under low compaction (Fig. 7b; $P < 0.05$ days 8–17). At both soil compaction levels, lateral roots emerged 11 days after sowing but already 3 days later growth of lateral roots was significantly reduced when soil compaction was moderately increased (Fig. 7c; $P =$
In total, lateral root systems of plants grown under 0.52 MPa were 34% shorter than those of plants grown under 0.06 MPa. In a similar range rooting depth was inhibited by soil strength. Until the end of observation (day 20) roots did not reach the bottom of the rhizotrons. Soil compaction affected not only the root growth rate, but also the spatial distribution of roots within the rhizotrons (Fig. 7d). The soil was homogeneously compacted within the rhizotrons, except for the top 5 cm, which were filled in both conditions – low and moderate compaction – with loose soil. We noted that plants grown in more compacted soil induced significantly root growth into this top soil layer ($P = 0.004$). However, below a depth of ~25 cm, root length density of plants grown in moderate compacted rhizotrons revealed a strong decrease. This reduction was significant in the horizon starting at 32 cm and including deeper soil layers (Fig. 7d; $P = 0.039$). In conclusion, these results highlight that the automated rhizotron cultivation system and the imaging routine enable detection of changes in root length and geometry of root systems caused by relatively moderate mechanical stresses.
2.5 Discussion

The novel method GROWSCREEN-Rhizo enables us to phenotype root systems and correlate root traits to whole-plant development

The novel phenotyping system presented here, which we named GROWSCREEN-Rhizo, is capable of delivering quantitative information on root-system development and plant performance of rhizotron-grown plants. This provides essential information to tackle biological questions stemming from both basic research as well as from breeding processes. For example, this method is applicable to detect differences in root-system
architecture induced by relatively moderate increases in soil compaction (Fig. 7). An increase in soil compaction from 0.06 to 0.52 MPa resulted in significant reduction in growth of main as well as lateral roots of barley plants (Fig. 7b, c). It has been reported for several species that root elongation rate varies inversely with soil resistance within a range from 0 to 7.5 MPa (e.g. Atwell 1993; Bengough et al. 2011). In our experiments, mechanical impedance due to compaction of the soil caused not only a reduction of root growth but also a spatial distribution of roots along the soil profile. An increase in soil strength resulted in a shift of root distribution to the top soil layers whereas rooting depth was decreased (Fig. 7d). These results obtained in soil-filled rhizotrons are in line with findings obtained in the field (Lipiec et al. 1991). Although root-system development was reduced under moderate soil compaction in our rhizotrons, leaf growth was unaffected (Fig. 7a). This is apparently in contrast to the findings by Beemster et al. (1996), who showed that resistance to root penetration leads to a reduction in leaf cell elongation of wheat plants, although leaf growth was more strongly affected than root growth (Masle 1992). The discrepancy between these studies and our findings can be explained by the much higher level of soil compaction (7.5 MPa) that Beemster et al. (1996) applied compared with the treatments in our experiments. Apparently, a certain threshold of soil resistance to root penetration has to be reached to affect leaf growth. This hypothesis is confirmed by Lipiec et al. (1991), who showed that high levels of soil resistance are needed to decrease leaf area index of barley plants grown in the field. However, the degree to which the reduction in root development triggered by mechanical impedance reduces shoot biomass or yield also depends on the extent of restriction in water and nutrient uptake (Clark et al. 2003).

The distribution of root length per unit volume in the soil profile is the key to extract sufficient water and nutrients (Gregory et al. 2009). Differences in root length density along the depth of rhizotrons were also detected when monocot and dicot species were screened (Fig. 4). The dicot species Arabidopsis and rapeseed exhibited a higher root length density in top substrate layers, but lower values were found in deeper layers compared with the monocot species, Brachypodium and barley. These modifications can be ascribed to morphological differences of monocot and dicot root system. The allorhizic root system of dicotyledons is characterised by the development of one primary root and lateral roots that start branching at the base of the root system (Osmont et al. 2007). Consequently, during the first weeks after germination, a higher root length density would be expected in top soil layers. Yet, in homorhizic root systems such as those of monocots,
many adventitious roots develop in parallel to the primary root (Osmont et al. 2007) and lead to a higher root length density in deeper layers.

In addition to non-invasive phenotyping of root systems GROWSCREEN-Rhizo offers the advantage to screen root and shoot growth simultaneously and correlate root traits to whole-plant development. The non-destructive analysis enables us to compare the impact of treatments at various reference stages, e.g. at the same leaf area size. Therefore, it is possible to distinguish whether a treatment affects the speed of development or has direct interactions with plant development. For dicotyledonous plants, like Arabidopsis or tobacco seedlings that have leaves which spread out almost horizontally at midday, projected leaf area development can be quantified automatically by acquiring images of leaves from the top view of the plants (Granier et al. 2006; Walter et al. 2007). Leaf growth of monocots such as barley and maize, can be estimated by images taken from different camera angles. We show that the projected leaf area correlated linearly with the shoot biomass of barley and maize plants ($R^2 > 0.95$; Fig. 5). Similar correlations were found previously by using a commercially available plant image capture and analysis system (Rajendran et al. 2009). Since these methods resulted in similar correlation coefficients ($R^2 = 0.94$ for wheat (Rajendran et al. 2009), $R^2 = 0.95$ for barley (Fig. 5c) and $R^2 = 0.98$ for maize (Fig. 5d) respectively), our imaging setup appears to be sufficient to estimate plant biomass as a linear function of the projected leaf area for the examined monocot species at early vegetative stages characterised by moderate overlap of different leaves. For further improvement in the accuracy of biomass estimation, Golzarian et al. (2011) presented a model for wheat and barley plants that integrates information obtained from the images with plant age. However, using projected shoot area as an estimator of shoot biomass requires validation for different species characterised by diverse shoot architecture and depending on different treatments simulating environmental scenarios.

*The fraction of the visible part of the root system in rhizotrons is correlated with the total root system and plant development*

Growing plants in rhizotrons facilitates non-invasive measurements of the same individual at frequent time intervals. However, even if roots are forced to grow towards the transparent plate by inclining rhizotrons, only a part of the root systems is visible and accessible for cameras (Fig. 6a; Tables 1, 2). The proportion of visible roots at the transparent interface of rhizotrons depends slightly on soil strength (Table 1) and can be
enhanced by increasing the inclination angle of rhizotrons (with the transparent side facing downwards). Consequently, to standardise protocols and achieve reliable comparisons between individual plants, it is necessary not only to ensure homogeneous filling of the rhizotrons but also to control their inclination angles. In addition, the percentage of visible roots varies between plant species (Fig. 6a; Table 2). The fraction of visible roots seems to be related to specific root weight and root diameter of plant species: the thinner the roots, the higher the percentage of visible roots: although a relatively large proportion of thin roots of Arabidopsis plants (root diameter ~100 mm; van der Weele et al. 2000) was visible (~77%), the smallest fraction of roots was visible (~17%, Table 2) when ~10 times thicker roots of maize plants (van der Weele et al. 2000) were observed in rhizotrons. Rapeseed, barley, rice and Brachypodium plants exhibited values ranging between those of Arabidopsis and maize plants (Table 2; e.g. Hargreaves et al. 2009; Watt et al. 2009). Kuchenbuch and Ingram (2002) reported similar results for maize (~20%) and Hurd (1964) showed for wheat plants that the visible root length represents ~30% of total root-system length. Consequently, the visible part of the root system can be used only as a measure for growth of total root system if differences between species are taken into consideration and well defined protocols are used. In addition, the assumption that the visible part is a constant fraction of the total root system must always be thoroughly checked before analysing new species or changing environmental conditions such as soil structure, soil water content or root zone temperature. Besides the correlation between the visible and the total root system, it is useful to address whether root and shoot growth profiles observed in rhizotrons are comparable with those detected in other growth media and conditions. Further studies are needed to test if the transparent plate of rhizotrons, along which roots are forced to grow, modifies root growth or root-system architecture and whether the root traits observed in rhizotrons are relevant under field situations. For this approach, field- and also agar-grown plants can be taken into account due to the visibility and accessibility of whole-root systems in transparent media. The combination of different methods and approaches under artificial and natural environments and the integration at different scales into ‘phenotyping chains’ will improve our knowledge of the hidden half of plants and will open novel routes for plant breeding (De Smet et al. 2012).
Table 3. Root traits measured non-destructively with the novel system GROWSCREEN-Rhizo of plant roots grown in rhizotrons. Broad sense heritability ($h^2$) values for certain root traits in literature are indicated ($n = 3–10$).

<table>
<thead>
<tr>
<th>Root traits</th>
<th>Primary data</th>
<th>Plant species</th>
<th>Heritability $h^2$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main root length/kinetics</strong></td>
<td>Length of main roots (cm)</td>
<td>Arabidopsis</td>
<td>0.44 (1 µM Zn) – 0.75 (250 µM Zn)</td>
<td>Richard et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wheat</td>
<td>0.42</td>
<td>Laperche et al. (2006)</td>
</tr>
<tr>
<td><strong>Lateral root length/kinetics</strong></td>
<td>Total length of branched roots (cm)</td>
<td>Arabidopsis</td>
<td>0.65 (1 µM Zn) – 0.44 (100 µM Zn)</td>
<td>Richard et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wheat</td>
<td>0.38</td>
<td>Laperche et al. (2006)</td>
</tr>
<tr>
<td><strong>Root-system length/kinetics</strong></td>
<td>Sum of all visible roots (main, shoot borne and lateral roots) (cm)</td>
<td>Cotton</td>
<td>0.99</td>
<td>Malik et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Potato</td>
<td>0.93 (control) – 0.84 (drought stress)</td>
<td>Anithakumari et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wheat</td>
<td>0.87 (control) – 0.84 (drought stress)</td>
<td>Dhanda et al. (2004)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soybean</td>
<td>0.69</td>
<td>Ao et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rice</td>
<td>0.64</td>
<td>MacMillan et al. (2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medicago truncatula</td>
<td>0.51 (control) – 0.44 (salt stress)</td>
<td>Arraoudi et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wheat</td>
<td>0.41</td>
<td>Laperche et al. (2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rice</td>
<td>0.41</td>
<td>Roy et al. (2009)</td>
</tr>
<tr>
<td><strong>Root length density/kinetics</strong></td>
<td>Ratio length of root system to surface area of rhizotrons (cm cm$^{-2}$)</td>
<td>Chickpea</td>
<td>0.14 – 0.57 depending on season and rooting depth</td>
<td>Kashiwagi et al. (2005)</td>
</tr>
<tr>
<td><strong>Depth of root system/kinetics</strong></td>
<td>Maximum vertical depth of whole root system (cm)</td>
<td>Soybean</td>
<td>0.53</td>
<td>Ao et al. (2010)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chickpea</td>
<td>0.36</td>
<td>Kashiwagi et al. (2005)</td>
</tr>
<tr>
<td><strong>Width of root system/kinetics</strong></td>
<td>Maximum horizontal width of whole root system (cm)</td>
<td>Soybean</td>
<td>0.62</td>
<td>Ao et al. (2010)</td>
</tr>
<tr>
<td><strong>Angle of shoot borne roots</strong></td>
<td>Angle between the horizontal and shoot borne roots (°)</td>
<td>Sorghum</td>
<td>0.47</td>
<td>Singh et al. (2011)</td>
</tr>
<tr>
<td><strong>Branching angle of lateral roots</strong></td>
<td>Angle between main and branched lateral roots (°)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Simple root morphological traits have higher heritability values compared with global architectural traits

The novel system GROWSCREEN-Rhizo enables the measurement of simple morphological traits (e.g. root length) and global architectural traits (e.g. width and depth of root system and root length density profiles) of root systems of different species (Figs 5–7; Table 3). The possibility of quantifying branching angles of lateral roots or angles in which main and shoot borne roots emerge in rhizotrons depends on the visibility of the branching/starting point of roots. Due to the fact that often parts of individual roots are hidden in the soil, quantification of the number of main, shoot-borne or lateral roots is challenging in rhizotron-grown plants. Since root-system architecture has not been well explored to date, it may be worth to measure as many root traits as possible. Scaling the novel system to a desired throughput and improving further the software for automated analyses of root systems will enable phenotyping of large numbers of genetic diverse genotypes. This is indispensable to evaluate the relevance of measured root traits and to find heritably traits correlated with resource use efficiency, performance and yield of plants. Especially, for breeding strategies, heritable traits play a key role. In contrast to root biomass, which appears to have low heritability values (Jones 1977), moderate and high heritability values were reported for root length of main and lateral roots as well as for total root systems (Table 3). The highest heritability values were found for root length of potato and cotton plants with $h^2$ of up to 0.99 (Anithakumari et al. 2011; Malik et al. 2011). Heritability was, in general, slightly lower under drought or salt stress than under control conditions (e.g. Dhanda et al. 2004; Anithakumari et al. 2011; Arraouadi et al. 2011). In the presence of Zn concentration ranging from 1 to 250 mM the broad sense heritability for primary and lateral root length of Arabidopsis accessions varied between 0.44 and 0.75 (Richard et al. 2011). Moderate heritability was found for nodal root angle (0.47; Singh et al. 2011), depth of root system (up to 0.53; Ao et al. 2010) and only slightly higher heritability values for width of root system (0.62; Ao et al. 2010). Based on these studies, root morphological traits have higher heritability values than global architectural ones and could be more valuable for breeding progress. For example, it could be difficult to breed for root length density because of the lowest heritability values and the largest range of variation across seasons and rooting depth ($h^2 = 0.14–0.57$) compared with other root traits (Kashiwagi et al. 2005). However, this literature survey highlights that, to date, heritability values of root-system architecture have been published
only for a few plant species; as a consequence, caution is necessary in making widely applicable generalisations. Further studies are required and these will accelerate the progress in prediction of genotypic and phenotypic effects during the selection of plant material (Johnson et al. 1955; Malik et al. 2011). Promising belowground features that should be addressed in breeding programs to improved water and nutrient uptake of plants are for example root growth, branching rate and root angle (Hammer et al. 2009; Herder et al. 2010; Lynch 2011). Optimising these root traits could lead to an increased yield production provided that the right balance in resource allocation between root and shoot is ensured (Lynch 2007).

2.6 Conclusion

The novel platform described in this work is a unique automated prototype to phenotype root-system architecture of a diverse set of plant species grown in soil-filled rhizotrons. The system provides a step towards bridging the gap between laboratory and field and enables to quantify static and dynamic characteristics of root systems and to correlate them to whole-plant growth and development. The evaluation of root traits of a diverse set of genetic resources under a range of environmental conditions will give the opportunity to discover the genetic control of root-system architecture. The prototype scaled to a desired throughput (thousands of plants) will represent a valuable tool to characterise gene function and assist breeding pipelines by selecting genotypes with improved plant growth performance, biomass and yield production.

2.7 Acknowledgements

We are indebted to Thorsten Brehm, Marcel Schneider, Beate Uhlig and Franz-Wilhelm Genzer for installing drainage and irrigation system of the GROWSCREEN-Rhizo setup. We are grateful to Saaten-Union BiotecGmbH for providing us with seeds of *Hordeum vulgare* cv. Golden promise. We thank Birgit Bleise, Anne Dreissen and Nadja Vöpel for technical assistance during harvest of rhizotron-grown plants. M. E. acknowledges the support by the BMBF Network CropSense, A. G. by the BMBF project Pre-BreedYield, S. B. by the European Community’s Seventh Framework Programme (grant no 226532) and J. P. by the German Research Foundation (FOR 1320).
Chapter 3

Spring barley shows dynamic compensatory root and shoot growth responses when exposed to localised soil compaction and fertilisation

3.1 Abstract

The impact of heterogeneous soil compaction in combination with nutrient availability on root system architecture and root growth dynamics has scarcely been investigated. We quantified changes of barley (*Hordeum vulgare* L.) root and shoot growth during the first 3 weeks of growth in a controlled-environment chamber. Vertically divided split-root rhizotrons were filled either uniformly with loose or compacted peat, or heterogeneously with loose peat in one compartment and compacted peat in the other. We investigated the following questions. (a) Can growth processes affected by soil compaction be mimicked in our system? (b) Do plants show compensatory growth effects when exposed to heterogeneous soil compaction? (c) Does localised fertiliser application affect root systems’ responses to compaction? We observed compensatory effects regarding root system architecture and root growth dynamics due to vertically heterogeneous soil compaction. Roots grew deeper and lateral roots emerged earlier in the loose compartment of the split-root treatment compared with uniform treatments. When fertiliser was applied only the compacted compartment in the split-root treatment, more lateral roots were initiated in the compacted compartment and lateral root formation started a few days earlier than in the uniform treatments. Consequently, the first days after exposure to heterogeneous soil conditions are critical for the analysis of underlying physiological responses.

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3.2 Introduction

Soil compaction is a serious agronomic problem since it leads to a range of negative effects on soil structure, often resulting in hampered growth of roots and shoots and, consequently, persistent yield decline (Håkansson and Reeder 1994; Chamen et al. 2003). Due to a decreased pore volume in compacted soil (the macropore volume is particularly affected), the capability to store and transport air and water is often reduced (Hamza and Anderson 2005) and therefore the mobility of nutrients by mass flow and diffusion can also be significantly decreased. These changes in the conductivity of air and water in compacted soil can result in a higher probability for hypoxia and water stress to the plant. Furthermore, strongly compacted soil typically leads to an increased mechanical resistance of the soil, which impedes root growth. A remarkable number of recent research efforts have focussed on the growth response of crops on soil compaction (e.g. Bingham and Bengough 2003; Grzesiak 2009; Kautz et al. 2010; Haling et al. 2011). Moreover, recent studies emphasise the need for selecting genotypes that are adapted to high soil compaction (e.g. Grzesiak 2009; Haling et al. 2011). Most research addressing the effects of soil compaction on plant growth performance have utilised homogeneously compacted soil in laboratory or greenhouse experiments (Konôpka et al. 2009). Konôpka et al. (2009) investigated maize (Zea mays L.) roots affected by structured field soil containing clods and fine soil in the laboratory, and observed several changes with respect to root morphology.

Soil compaction is often heterogeneously distributed in agricultural fields. Routine soil cultivation by ploughing frequently leads to so-called pan layers in the horizontal direction, whereas wheel tracks form heterogeneously compacted structures in the vertical direction (Soane et al. 1982; Chamen et al. 2003; European Soil Portal 2013). These effects are often visible as poor aboveground growth of crop stands (Wolfe et al. 1995). The impact of heterogeneously distributed soil compaction on root system architecture (RSA) and especially root growth dynamics has scarcely been investigated either at the field or laboratory scales, mainly due to technical reasons.

Most methods on the field and laboratory scale that use soil as growth medium are labour-intensive; expensive; allow only a very low throughput, such as X-ray computed tomography (Tracy et al. 2012) or nuclear magnetic resonance imaging (Jahnke et al. 2009); are destructive (shovelomics, Trachsel et al. 2011); or are not applicable to
quantify RSA traits (such as minirhizotrons (Polomski and Kuhn 2002)). Root research requires noninvasive monitoring tools for the investigation of the phenotypic traits that enable the dynamic measurement of RSA that has been affected by different soil conditions (physical and chemical), as described by Nagel et al. (2012).

In the context of this study, the dynamic responses of root systems to localised soil compaction and fertilisation have been investigated noninvasively by growing barley (*Hordeum vulgare* L.) plants in vertically divided split-root rhizotrons. The aim was to quantify to what extent plants are able to ‘compensate’ for the growth of the shoot when parts of the root system are exposed to less favourable conditions compared with control plants. Compensation could occur because of transient increases in root uptake and may be related to plastic responses in root placement as well as in root biomass. Would such responses result in increased root growth in the less compacted volumes of a heterogeneously compacted soil substrate? Would these effects be accompanied by measurable differences in shoot photosynthetic rates, carbon gain and the mineral content of leaf tissue?

The time scales at which acclimation to altered environmental conditions occurs differ widely. With respect to soil compaction, it has been observed that leaf elongation can be inhibited within a few minutes after the root system has experienced increased mechanical impedance (Young et al. 1997). Alterations in the intensity of lateral root formation due to different soil compaction levels have only been indicated for a longer time scale of more than 1 week (Tracy et al. 2012). Lateral root formation is an important means for the plant to adjust to heterogeneous situations in the soil: increased formation of lateral roots in favourable patches of soil characterised by higher water or nutrient availability can be a decisive advantage. Knowledge concerning the time scale of these processes is required to design experiments that allow the identification of the mechanisms controlling the alteration of RSA or that allow the identification of favourable crop genotypes in breeding programs. Laboratory studies investigating the effect of vertically localised soil compaction are rare but are urgently needed (Bingham and Bengough 2003). In the present study, we investigated the ability of spring barley to respond to vertically localised compaction in terms of root and shoot development, as well as in terms of RSA. To date, it is unclear how rapidly RSA can respond to alterations of soil compaction, drought or other soil properties that inhibit growth and development. The aim of this study was to investigate the following questions. (a) Can root and shoot growth processes that have been affected by soil compaction be mimicked in our system?
(b) Do plants show compensatory root and shoot growth effects when roots are exposed to heterogeneous soil compaction? (c) Does localised fertilisation have an effect on root systems’ responses to compaction?

To address Question (c), we applied fertiliser exclusively to the compacted compartment in the heterogeneous split-root treatment. We assume that this nutrient supply is disadvantageous in comparison with the split-root treatment in which fertiliser was applied also in the compartment filled with loose soil.

### 3.3 Materials and methods

**Plant material and cultivation conditions**

Spring barley seeds (*Hordeum vulgare* cv. Golden Promise) were germinated at 21°C in Petri dishes (Greiner Bio-One, Frickenhausen, Germany) on filter paper that was moistened with deionised water (Milli-Q, Millipore Corporation, Billerica, MA, USA). Three days after germination, seedlings with six seminal roots each (~4 cm long) were selected and transplanted into rhizotrons (Fig. 1a; (rhizotrons were constructed in cooperation with the company Metallbau Mäsgen, Volmershofen, Germany)). We cultivated plants using rhizotrons (80 cm × 30 cm × 2 cm) that were divided by a septum into two equal compartments 14.5 cm wide. One seedling was placed in the centre of the rhizotron just above the septum. For each seedling and treatment, the root system was divided into two halves, where three seminal roots were placed in each compartment of the rhizotron for a total of five replicates.
The rhizotrons were filled with black peat soil (Florabella Tuintrof, Klasmann-Deilmann, Geeste, Germany) manually sieved to a maximal aggregate size of 0.5 cm. To adjust the pH of the substrate to 4.5, the soil was uniformly mixed with powdered gardening lime (Dolomag 95 kohlensaurer Kalk 95, 95% CaCO3, 53% CaO, containing trace elements, (a) From left to right, drawings illustrate the four treatments which were compared: UL (uniform loose), UC (uniform compacted), SC (split compaction) and SCSF (split compaction and split fertilisation). (b) Typical colour-coded image of roots visible at the transparent plate of rhizotrons with seminal roots in green and lateral roots in red analysed with the software GROWSCREEN-Root.
Rheinkalk KDI, Wülfrath, Germany). The final mix contained, relative to the total composition of dry peat mass (mg kg\(^{-1}\)): 81 mg kg\(^{-1}\) N comprising 75 mg kg\(^{-1}\) NO\(_3\) and 6 mg kg\(^{-1}\) NH\(_4^+\); <12 mg kg\(^{-1}\) P\(_2\)O\(_5\); 149 mg kg\(^{-1}\) K\(_2\)O; 3478 mg kg\(^{-1}\) Mg; 17 mg kg\(^{-1}\) Mn (electrical conductivity, 530 µS cm\(^{-1}\); dry bulk density, 0.161 kg L\(^{-1}\); dry matter content, 31%; fresh density, 520 g L\(^{-1}\)). Peat was used because it is a relatively nutrient-poor substrate and hence the availability of certain nutrients can be adjusted to a large extent by supplying plants with a nutrient solution. Moreover, the black colour of the peat improves the optical contrast between roots and soil and therefore simplifies the measurement of roots in image analysis. To achieve standardised compaction across replicate split-root rhizotrons, we used a protocol that has been described previously (Nagel et al. 2012). Here, 300-g portions of fresh substrate were poured gradually into the rhizotrons and subsequently compressed using a custom-built compaction frame. The compression was applied by a manual pallet forklift by lifting individual rhizotrons against the frame while a defined pressure to the soil surface was applied by means of a wooden plank. Applied pressure and compaction values were calculated using a scale. Two draining drills with a diameter of 0.8 cm at the bottom of the rhizotrons maintained sufficient drainage and oxygen supply to the roots. Due to the compaction of the soil in layers, the variation in the soil strength over depth is negligible.

To keep the soil water content at a minimum of 50% volumetric water content and to ensure sufficient water supply, plants were watered every other day with 50 mL of deionised water. Rhizotrons were kept at an inclination angle of 45° in a controlled environment chamber. The experimental design was completely randomised. The transparent plates of the rhizotrons were facing downwards and were covered with black plastic foil to prevent light reaching the roots. The air temperature was maintained at 21°C ± 0.5°C day and night with a photoperiod of 12 h : 12 h light : dark. Relative air humidity was maintained at 65% ± 10%. Illumination was supplied by fluorescent tubes (Osram L 58W or 77 Fluora 58 W, Osram GmbH, Munich, Germany) that provided 240 µmol m\(^{-2}\) s\(^{-1}\) PAR measured at the initial plant height.

**Treatments**

Four treatments were applied, where the different compartments of the split-root rhizotrons were filled either with densely compacted substrate (hereafter called the compacted substrate) or loosely compacted substrate (hereafter called the loose substrate;
The dry bulk density of the compacted substrate (measured in additional monitoring rhizotrons; see below) was 0.28 ± 0.006 g mL\(^{-1}\) with a penetrometer resistance of 1.23 ± 0.11 MPa and the bulk density of loose substrate was 0.18 ± 0.005 g mL\(^{-1}\) with a penetrometer resistance of 0.05 ± 0.01 MPa. The pore volume was 0.80 ± 0.004 mL mL\(^{-1}\) in the compacted substrate and 0.87 ± 0.004 mL mL\(^{-1}\) in the case of loose substrate. The pore volume filled with air (air-filled porosity) was 0.15 ± 0.01 mL mL\(^{-1}\) in the compacted substrate and 0.39 ± 0.02 mL mL\(^{-1}\) in the case of loose substrate. All physical soil measurements were conducted at harvest with a replicate number of \(n = 15\) soil samples.

For testing Questions (a) and (b), plants grown in rhizotrons filled with loose substrate in one compartment and compacted substrate in the other compartment of the split-root rhizotron (the split compaction (SC) treatment) were compared with plants grown in rhizotrons filled either with loose substrate in both compartments of the split system (the uniform loose (UL) treatment) or compacted substrate in both compartments of the split system (the uniform compacted (UC) treatment; Fig. 1a).

For the entire duration of the experiment and for each treatment, each individual plant was given fertiliser to meet 100% of the expected demand and nutrient removal over the lifetime of a typical spring barley plant as recommended by the German fertilizer regulation as described by the Thüringer Landesanstalt für Landwirtschaft (TLL 2005; Düngeverordnung 2012). The nutrient removal values listed by the German Fertilisation Edict (Düngeverordnung (DÜV)) are the amounts of a nutrient that a plant will take up from germination until harvest. The DÜV suggests not applying more than this amount of fertiliser. We used these values to have an estimation of an appropriate nutrient supply. Therefore both split-root compartments of the rhizotrons were each given 50% of the nutrient removal as fertiliser. Nutrients were supplied to each fertilised compartment via a nutrient solution (1.22 mmol L\(^{-1}\) N, 0.13 mmol L\(^{-1}\) P, 0.11 mmol L\(^{-1}\) K and 27.9 nmol L\(^{-1}\) Si) at three dates: 25% of nutrient removal was given at the day of transplanting, 12.5% was given 7 days after transplanting (DAT) and another 12.5% was given at 14 DAT. Manganese fertilisation was performed by spraying the leaves at 7 DAT with 2 mg Mn per plant (MnSO\(_4\) solution, 1.3 g L\(^{-1}\)).

To test Question (c), an additional treatment (the split compaction and split fertilisation (SCSF) treatment) was compared with the other ones. Rhizotrons were filled in the same way as the SC treatment but fertiliser was applied only over the compacted compartment with twice the amount of nutrient solution in comparison with the compartments under
the SC treatment. Therefore, 100% of the assumable demand was applied at the same
dates but here, 100% was given to the compacted side at the same points in time using
three volumes of a nutrient solution containing 50%, 25% and 25% of the total,
respectively. The loose side of the SCSF treatment (named L(SCSF)) received an equal
amount of deionised water. Whenever the compacted side of the SCSF treatment (named
C(SCSF)) was given the nutrient solution, all differences in water supply compared with
this treatment were compensated accordingly to keep the soil water content comparable in
all treatments.

**Phenotyping of RSA and analysis of plant growth and biomass**

Data acquisition was performed manually using a custom-made photo-box. Images of the
root systems were acquired using a single-lens reflex camera (SLR) camera (10 Mpx,
with a 28 mm electro focus lens (EFS) lens, EOS digital 400D; Canon U.S.A., Inc.,
Melville, NY, USA). The resolution of the acquired images was adequate for detection of
semenal and lateral roots. The photo-box was equipped with a black cardboard
background with a hole through which the camera lens was positioned to minimise
reflections on the transparent plates of the rhizotrons. The custom-made image analysis
software GROWSCREEN-Root (Institute of Bio- and Geosciences, Plant Sciences (IBG-2),
Forschungszentrum Jülich GmbH, Jülich, Germany) was used to quantify the length of
semenal and lateral roots, maximal rooting depth and root length density (Fig. 1b, Nagel
et al. 2012). In addition, we computed the 2D convex hull (the smallest convex polygon
enclosing all root pixels) as a geometric parameter describing the coverage of the root
system visible at the transparent rhizotron plate.

Nagel et al. (2012) determined the correlation between the visible portion of the root
system quantified by image analysis and the total root length measured destructively in
plants of *H. vulgare* cv. Golden Promise (the same cultivar as used in this study) grown at
low (0.30 MPa) and moderate compaction (0.78 MPa). For both compaction levels, the
portion of the total root length that was visible at the transparent plate of the rhizotrons
was very similar, namely 29.4% ($R^2 = 0.87$) and 27.2% ($R^2 = 0.72$) under low and
moderate compaction, respectively. These data show that, at least for early vegetative
stages of this barley cultivar, a moderate increase in soil mechanical impedance does not
affect the portion of the root system visible at the transparent plate of rhizotrons.
Images of the shoots were acquired using two cameras (Canon EOS digital 400D) mounted at an angle of 90° to each other on top of the photo-box. The background was equipped with black cardboard. The projected leaf area was determined using custom-made image analysis software (Nagel et al. 2012) by averaging the leaf area from the two sides.

At 21 DAT, shoots were harvested and dried for 3 days at 70°C in a drying oven (Heraeus UT 6760, Thermo Scientific Heraeus, Langenselbold, Germany) to measure shoot biomass. Leaf area was additionally determined directly after harvest using a leaf area meter (LI-COR Biosciences, Lincoln, NE, USA) for the calculation of specific leaf area (Table 1). At the same time, roots were washed out, and total root length and root diameter classes were determined using WinRHIZO (images with 400 dpi; Regent Instruments Inc., Sainte-Foy, Québec, Canada). Moreover, root DW was measured after drying for 3 days at 70°C. Parameters like root surface area and root volume were calculated with values obtained with WinRHIZO. Scanned root images were used to quantify the number of lateral roots by manually labelling and counting them using ImageJ software (ImageJ, National Institutes of Health, Bethesda, MD, USA).

Quantification of substrate chemical and physical parameters, shoot nutrient concentration and leaf gas exchange

Three additional split-root rhizotrons were filled as controls with the substrate being analogous to the UL, UC and SC treatments for measurement of soil bulk density and mechanical resistance. These rhizotrons were placed in the same growth chamber and treated exactly as the other ones regarding transplanting of seedlings, fertilisation and irrigation. Samples of undisturbed substrate were taken using soil cylinders (volume: 10.6 mL). The DW of soil samples was determined after 48 h drying at 105°C. Substrate mechanical resistance (penetrometer resistance) was measured in these additional monitoring rhizotrons just before harvest by a hand penetrometer for top layers (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands) that had a 30° cone angle, an 8 mm maximal cone diameter and a penetration depth of 10 cm, and was fitted with 5-N and 50-N steel springs.

Soil samples (100 g, n = 3–6) for nutrient analysis were taken from three randomly selected rhizotrons for each treatment at depths of 21 cm, 42 cm and 63 cm, and they
were subsequently air-dried. After determination of DW, shoot samples collected at 21 DAT (see above) were pulverised using a swing mill (Retsch MM200, Retsch GmbH, Haan, Germany). The substrate and shoot samples were sent to the BayCEER laboratory (University of Bayreuth, Germany) for measurement of the nutrient concentration (extraction method after the German standard: calcium chloride DTPA (diethylene triamine pentaacetic acid) solution in the CAT LUFA method (LUFA: Landwirtschaftliche Untersuchungs- und Forschungsanstalt) (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten 2013), inductively coupled plasma optical emission spectrometry (ICP-OES), the C : N ratio and, in the case of substrate, pH value (after the German standards (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten 2013)). Nutrient concentrations from the shoot tissue were interpreted using the sufficiency ranges approach (Plank and Donohue 2000) to assess whether nutrients were supplied in sufficient quantities. To assess potential nutrient imbalances in greater detail, an adapted Diagnosis and Recommendation Integrated System (DRIS) was used in addition to the sufficiency ranges approach. DRIS was originally developed as a diagnostic tool for the detection of essential elements limiting yield (Beaufils 1957). A DRIS index can be calculated for each nutrient element using several nutrient concentration ratios that are related to certain DRIS norm ratios. DRIS norm ratios are used as a reference (here, we used the greatest 25% of shoot DW; see below). The optimum DRIS index for any nutrient element is 0.0. Negative DRIS indices indicate deficiency and positive indices indicate sufficiency. Therefore, the DRIS approach goes further than the sufficiency ranges approach and answers, under certain conditions, the question of which specific element is limiting yield (in our case, this was assessed by means of shoot biomass) and is supplied in relative deficiency. The sufficiency ranges approach commonly cannot provide information about which element is the most limiting. In our experiment, DRIS indices were calculated on the basis of the nutrient concentrations in the shoot tissue using the arithmetic mean value of the greatest 25% of shoot DW as the DRIS norm in the equation, according to Sumner (1981) and Jones (1981). This means, in our case, that the indices are relative to these 25% of plants with the highest shoot DW. The DRIS index for the particular nutrient element and treatment can be interpreted as being deficient for achieving a high shoot mass (compared with the top 25% in our experiment) when the DRIS index is negative, for example.

At 17 DAT, after irrigation at 0900 hours, photosynthetic assimilation and stomatal conductance were measured on the third youngest leaf of all individual plants (plants had
three to five leaves at that time and the investigated leaf was fully expanded) using a LI-COR 6400 portable photosynthesis measurement system (LI-COR Biosciences, Lincoln, NE, USA).

Statistical analysis

Differently treated compartments of the four treatments were compared as shown in Fig. 1a. Compaction level and treatment are specified by letters and abbreviations: a letter before parentheses refers to the compaction level within the respective treatment (e.g. L(SC) denotes a parameter measured in material from the loose compartment within the SC treatment; see Fig. 1a). In the case of a comparison of both compartments within the same split-root treatment, it cannot be excluded that both compartments were statistically dependent from each other. Due to this reason, linear mixed models with the factor ‘rhizotron’ (= ‘individual plant’) were used by means of the SPSS ver. 17.0 statistical software package (IBM SPSS Statistics, International Business Machines Corporation (IBM), Armonk, NY, USA). Independent variables comparing the different treatments were analysed with one- or two-way ANOVA. In all cases where normality or homogeneity in variance were not achieved (or neither was achieved), non-parametric Kruskal–Wallis tests and Friedman tests were used for independent or matched data, respectively.

3.4 Results

Root and shoot biomass development

At harvest, 21 DAT, the shoot DW of plants from the UC treatment and from the SCSF treatment were significantly reduced (by 19% and 16%, respectively) in comparison with plants from the UL treatment (Fig. 2a). In contrast, the shoot DW of plants from the SC treatment was comparable with that of plants from the UL treatment (Fig. 2a). Similar relations were obtained for the leaf area of plants from the different treatments throughout their entire development. At harvest, several other plant traits were analysed, such as the number of leaves (Fig. 3b), the number of tillers, specific leaf area and root : shoot ratio
With respect to developmental traits, plants from the different treatments showed different relations: similar to the responses of shoot biomass, plants grown under the UL treatment developed a similar number of leaves and tillers as plants under SC, whereas plants from UC and SCSF developed less leaves and tillers than those under UL and SC. For specific leaf area and root : shoot ratio, the relations were similar: plants from the UC treatment had the smallest value and plants from the UL and SC treatments were comparable (Table 1). However, for the latter parameters plants from the SCSF treatment also maintained high values that were comparable to those from UL and SC.

**Table 1.** Effect of localised soil compaction and fertilisation on growth parameters at 21 days after transplantation. Different characters next to values indicate statistically significant differences (*P* < 0.05; one-way ANOVA; post hoc test: LSD method; arithmetic means and s.e. (in parentheses); n= 5).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uniform loose (UL)</th>
<th>Uniform compacted (UC)</th>
<th>Split compaction (SC)</th>
<th>Split compaction &amp; split fertilization (SCSF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tillers</td>
<td>1.2 (±0.25)</td>
<td>1.0 (±0.55)</td>
<td>1.2 (±0.58)</td>
<td>0.6 (±0.24)</td>
</tr>
<tr>
<td>Number of leaves</td>
<td>7 (±0.4) &lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6 (±0.4) &lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7 (±0.6) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>6 (±0.2) &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leaf width (mm)</td>
<td>7.4 (±0.62)</td>
<td>6.1 (±0.46)</td>
<td>6.4 (±0.55)</td>
<td>6.3 (±0.27)</td>
</tr>
<tr>
<td>Specific leaf area (m&lt;sup&gt;2&lt;/sup&gt; kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>29 (±0.8)</td>
<td>27 (±0.4)</td>
<td>29 (±0.8)</td>
<td>29 (±1.0)</td>
</tr>
<tr>
<td>Root area : leaf area ratio (cm&lt;sup&gt;2&lt;/sup&gt; cm&lt;sup&gt;-2&lt;/sup&gt;)</td>
<td>0.9 (±0.06) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6 (±0.02) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.7 (±0.06) &lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.8 (±0.07) &lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Root : shoot ratio (g g&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.30 (±0.016) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19 (±0.006) &lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.25 (±0.014) &lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26 (±0.029) &lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The root DW of plants grown in compacted soil in the compartments under UC, C(SC) and C(SCSF) were significantly reduced in comparison with the DW of roots grown in uniformly loose soil in the compartments of the UL treatment (Fig. 2b; 46%, 33% and 46%, respectively). In contrast, the root DW of plants grown in loose soil in the compartments under UL, L(SC) and L(SCSF) were significantly higher compared with
the DW of roots grown in the compartments under the UC treatment (185%, 162% and 169%, respectively). Plants from the SC and SCSF treatments did not differ with respect to root DW. It should be noted that the sum of the average root DW of one compartment of each of the uniform treatments (UL and UC; 0.024 + 0.013 = 0.037) equalled the sum of the root DW of the L(SC) and C(SC) compartments (0.021 + 0.016 = 0.037) as well as the sum of the L(SCSF) and C(SCSF) compartments (0.022 + 0.013 = 0.035). This means that it did not matter for root biomass production if the compacted compartment was integrated in a uniform split-root treatment (with another compacted compartment) or if the compacted compartment was integrated in a heterogeneous split-root treatment (with a compartment filled with loose soil in the other half of the rhizotron). The sum of the root DW from two compacted compartments and two loose compacted compartments was constant in our experiment, no matter how these compartments were distributed between treatments. Furthermore, this shows that root DWs from compartments with loose or compacted soil were comparable, independent from treatment. Consequently, differences in shoot growth between plants from the SC and SCSF treatments cannot be explained by root biomass data in the present experiment.
Leaf area, leaf number (Fig. 3a, b) and, as a trend, leaf width (Table 1) showed differences among treatments that were comparable to the ones described above for final shoot biomass. High values were reached in plants from the UL treatment and low values in plants from the UC treatment.

Figure 2. Effect of localised soil compaction and fertilisation on root and shoot biomass. (a) Shoot and (b) root DW at 20 days after transplantation. Treatments are labelled with the abbreviations UL (uniform loose), UC (uniform compacted), SC (split compaction) and SCSF (split compaction and split fertilisation). The first letter before the parentheses in (b) refers to compaction within each treatment (e.g. L(SC) denotes the loose compartment within the SC treatment). All values show the mean of one compartment. Different characters above bars indicate statistically significant differences ($P < 0.05$; shoot: one-way ANOVA; root: linear mixed models; post hoc test: LSD method; arithmetic means ± s.e.; $n = 5$).
With respect to the development of leaf area (Fig. 3a), the differences between the treatments were not modulated markedly during the time frame of observation. Plants from the UL treatment, for example, had the highest leaf area at the beginning and at the end of the experiment. Plants grown under SC showed an accelerated growth with respect to leaf area between 17 DAT and 20 DAT, and had a similarly increased rate of leaf appearance (Fig. 3b).

Root length showed clear differences between treatments (Fig. 3c). By the end of the experiment, compaction led to pronounced differences for total visible root length, whereas the application of fertiliser did not result in pronounced differences (Fig. 3c).

Total visible root length in the loose compartments (UL, L(SC) and L(SCSF)) was significantly greater than total visible root length in compacted compartments (UC, C(SC) and C(SCSF)) (Fig. 3c). The total visible root length in the UL treatment, for example, was 164% higher than the total visible root length in the UC treatment. In the L(SC) and L(SCSF) compartments, total visible root lengths were comparable. Also, total visible root length in the C(SC) and C(SCSF) treatments were comparable. Differences among treatments did not become more pronounced during the course of the analyses between 6 DAT and 20 DAT. Similar results were found for visible seminal (Fig. 3d) and lateral (Fig. 3e) root length among treatments as described above for total root length at the end of the experiment. However, during the course of the experiment, the relations between treatments changed, especially for the case of lateral root length (Fig. 3e). These dynamic changes can be elucidated in more detail by analysing ratios between lateral and seminal root length (Fig. 4). In particular, the strong increase in lateral root length in contrast to seminal root length in the compacted compartments of the split-root treatments (C(SC) and C(SCSF)) are much more visible in the ratio (Fig. 4) than in the absolute value of lateral root growth (Fig. 3e).

In order to compare the relations between root and shoot growth over time, we calculated the ratio between visible root length and projected leaf area for the four treatments (Fig. 5). Plants from the UL treatment showed a rather constant ratio from the beginning to the end of the experiment. In contrast, all treatments containing compacted substrate (UC, SC, SCSF) showed a decreasing ratio (indicating less growth with respect to root length compared with leaf area), especially from 10 DAT to 20 DAT, whereas the UC treatment showed the strongest decrease. Plants from the SC and SCSF treatments, where only part of the root system was growing in compacted substrate, were not significantly different in
their development between 17 DAT and 20 DAT from either UL or UC, but were intermediate between both uniform treatments (UL and UC) (Fig. 5).

Finally, root length was analysed by WinRHIZO at 21 DAT taking into account all roots that had grown in the entire volume of the rhizotron and not only those along the transparent window plates of the rhizotrons (Fig. 3f). Overall relations among treatments were similar to the ones described above for the last measurement obtained with GROWSCREEN-Root (Fig. 3c).

### Table 2. Effect of localized soil compaction and fertilization on root morphological parameters, at 21 DAT: root surface area (RA\textsubscript{surface}), convex hull area (CH), number of lateral roots in entire compartments (NOL\textsubscript{whole}) and in upper third of compartments (NOL\textsubscript{upper}; 0 – 27 cm depth). The number of lateral roots represents roots of first- and second-order lateral. Different characters next to values indicate statistically significant differences (P < 0.05; linear mixed models; post hoc test: LSD method; arithmetic means and s.e. (in parentheses); n=5). UL, uniform loose treatment; UC, uniform compacted treatment; L(SC), loose compartment in the split compaction treatment; C(SC), compacted compartment in split compaction treatment; L(SCSF), loose compartment in the split compaction and split fertilisation treatment; C(SCSF), compacted compartment in the split compaction and split fertilisation treatment.

<table>
<thead>
<tr>
<th></th>
<th>UL</th>
<th>UC</th>
<th>L(SC)</th>
<th>C(SC)</th>
<th>L(SCSF)</th>
<th>C(SCSF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA\textsubscript{surface} (cm\textsuperscript{2})</td>
<td>21 (±1.7)  \textsuperscript{a}</td>
<td>11 (±0.6)  \textsuperscript{d}</td>
<td>18 (±2.3)  \textsuperscript{ab}</td>
<td>14 (±1.3)  \textsuperscript{bcd}</td>
<td>17 (±2.5)  \textsuperscript{abc}</td>
<td>12 (±0.7)  \textsuperscript{cd}</td>
</tr>
<tr>
<td>CH (cm\textsuperscript{2})</td>
<td>693 (±60.2) \textsuperscript{a}</td>
<td>320 (±25.5) \textsuperscript{b}</td>
<td>807 (±40.0) \textsuperscript{a}</td>
<td>303 (±40.9) \textsuperscript{b}</td>
<td>671 (±73.0) \textsuperscript{a}</td>
<td>313 (±121.3) \textsuperscript{b}</td>
</tr>
<tr>
<td>NOL\textsubscript{whole}</td>
<td>704 (±54.3) \textsuperscript{a}</td>
<td>295 (±23.9) \textsuperscript{c}</td>
<td>531 (±73.5) \textsuperscript{bc}</td>
<td>383 (±52.2) \textsuperscript{bc}</td>
<td>494 (±86.7) \textsuperscript{bc}</td>
<td>449 (±54.0) \textsuperscript{bc}</td>
</tr>
<tr>
<td>NOL\textsubscript{upper}</td>
<td>507 (±41.6) \textsuperscript{a}</td>
<td>275 (±22.3) \textsuperscript{c}</td>
<td>313 (±55.3) \textsuperscript{bc}</td>
<td>335 (±41.8) \textsuperscript{bc}</td>
<td>307 (±46.7) \textsuperscript{bc}</td>
<td>431 (±57.6) \textsuperscript{ab}</td>
</tr>
</tbody>
</table>

**Spatial distribution of roots**

All roots growing in compartments filled with loose substrate (UL, L(SC) and L(SCSF)) grew significantly deeper and faster than roots growing in compartments filled with compacted substrate (UC, C(SC) and C(SCSF); Fig. 6a). Rooting depth of roots under L(SC) was significantly greater than rooting depth under UL (47% at 6 DAT; 26% at 15 DAT) and in all compacted compartments. Roots under L(SCSF) did not grow as deep as those under L(SC) from 6 DAT to 17 DAT. At 20 DAT, all roots under L(SC) reached
the bottom of the rhizotron and grew horizontally along the bottom (Fig. 6a). The depth to which roots grew under C(SCSF) was lower than in loose compartments and in C(SC). In the lower third of the C(SCSF) compartment, not a single root was found when roots were washed out of the soil. The distribution of root length density over depth (Fig. 6b) shows that densities in compartments filled with loose soil were high down to a depth of 80 cm, whereas the UC treatment, for example, did not result in high root length densities in zones deeper than 50 cm. Compartments filled with compacted soil from the heterogeneously filled split-root treatments (SC and SCSF) showed a slightly higher root length density at a depth of 60 cm.

*Growth dynamics of different root orders*

At 13 DAT, the ratio between lateral and seminal root length – visible at the transparent window plate of the rhizotrons – within the L(SCSF) compartment was significantly greater than that of roots within C(SCSF), and greater than those in the uniform UL and UC treatments (Fig. 4). At 15 DAT, the ratio within the L(SC) compartment was significantly greater in comparison with C(SCSF) and the compartments of the uniform UL and UC treatments. For the UC treatment, at 15 DAT, we could not observe any lateral roots and, in the case of UL, only very few. At 17 DAT, C(SC), C(SCSF) and UL showed pronounced lateral root growth activity, but in UC, very few laterals were visible. At 20 DAT, the greatest value for the ratio between visible lateral root length and visible seminal root length was observed for roots under L(SC). However, the value was not significantly different compared with all other treatments, except for roots from the UC treatment, which had the smallest ratio.
Figure 3. Effect of localised soil compaction and fertilisation on root and shoot growth dynamics. Development of (a) projected leaf area, (b) number of leaves, (c) total visible root length measured by GROWSCREEN-Root, (d) visible seminal root length measured by GROWSCREEN-Root, (e) visible lateral root length measured by GROWSCREEN-Root, and (f) total root length measured by WinRHIZO on washed out roots, 21 days after transplanting. Treatments are labelled with the abbreviations UL (uniform loose), UC (uniform compacted), SC (split compaction) and SCSF (split compaction and split fertilisation). The first letter before parentheses refers to compaction within each treatment (e.g. L(SC) denotes the loose compartment within the SC treatment). In (c) to (f), all values show the mean of one compartment. Different characters indicate statistically significant differences ($P < 0.05$; linear mixed models; post hoc test: LSD method; arithmetic means ± s.e.; $n = 5$).
At harvest, average root diameters were quantified using WinRHIZO. The average diameters of roots growing in compacted soil were significantly increased (Fig. 7a). Significant differences were found between plants grown under the split-root SC and SCSF treatments (Fig. 7a). The diameter classes observed in the present experiment were consistent with data published elsewhere (Drew et al. 1973; Goss 1977). Drew et al. measured root diameters for different root orders for the spring barley cultivar Proctor as follows: first-order laterals, 0.152–0.382 mm; second-order laterals, 0.138–0.206 mm; seminals, 0.364–0.532 mm. For the barley cultivar Proctor as well, Goss measured the diameter classes as follows: first-order laterals, ~0.150 mm; seminals, ~0.410 mm. These data from Drew et al. and Goss led us to the assumption that it might be possible to differ between seminal and lateral roots by means of the root diameter and measure seminal root length and lateral root length in this way. Shierlaw and Alston (1984), for example, observed that the diameters of first-order lateral roots were increased in ryegrass (Lolium perenne L.) and maize, but not those of second-order laterals when roots grew in compacted soil. In our experiment, the distribution of root order could adequately be estimated by use of the diameter classes. The determined diameter thresholds for second-order laterals (0–0.3 mm) were valid for roots growing in compacted substrate as well as roots growing in loose substrate in all samples analysed in the present experiment (data not shown). In the C(SCSF) compartment, which contained compacted soil that had two applications of fertiliser, significantly more thin roots (0–0.3 mm, representing second-order lateral roots) and significantly less thick roots (0.3–0.6 mm, representing seminal and nodal roots) were determined in comparison with the other compartments of the experiment that were filled with compacted soil (UC and SC; Fig. 7a, b). Plants within the C(SCSF) compartment produced significantly fewer significantly fewer seminal+nodal roots and significantly more second-order lateral roots in relation to total root length in comparison with those under C(SC) and UC (Fig. 7b, c). This result was consistent with the number of lateral roots (first- and second-order; Table 2) measured at harvest by optical assessment. The values of the average root diameter (Fig. 7a) were also significantly different for the roots in the different compartments but could not explain whether the relatively small root diameter in C(SCSF) (compared with the other compartments filled with compacted soil) was due to more thin roots or fewer thick roots,
or a change in the proportion of lateral root number or length. In order to determine whether it was lateral root length or number that was affected by the treatments, we counted lateral roots with ImageJ (Table 2). This analysis revealed that, as a trend, lateral root number was increased in the C(SCSF) compartment, particularly in the upper part of the rhizotron (Table 2).

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**Figure 4.** Effect of localised soil compaction and fertilisation on the ratio between lateral and seminal root length (cm) visible at the transparent plate of the rhizotrons. (a) Uniform loose (UL) and uniform compacted (UC) treatments; (b) split compaction treatment (SC); (c) split compaction and split fertilisation (SCSF) treatment. The first letter before parentheses refers to compaction within each treatment (e.g. L(SC) denotes the loose compartment within the SC treatment). All values show the mean of one compartment. Different characters indicate statistically significant differences ($P < 0.05$; linear mixed models, post hoc: LSD method; arithmetic means ± s.e.; $n = 5$).
Plants were sufficiently supplied with all macronutrients. This is shown by the DRIS indices approach (Table 3) as well as the critical level or sufficiency ranges approaches (Plank and Donohue 2000), the nutrient concentrations for which are shown in in Table 4; both are based on nutrient concentrations in shoot tissue. Using this interpretation framework, one can state that most nutrients were available in excess (except for Ca, Mg and Mn, which were available in sufficient quantities).

At harvest, the nutrient concentration in the shoot tissue of plants under the SC treatment was not significantly different to those under UL treatment, except for the P concentration, which was, as a trend, highest for SC, whereas the UC treatment had a significantly lower nutrient concentration in shoot tissue in comparison with the UL treatment in the cases of K and Na (Table 4).

For N and K total nutrient uptake (the sum of uptake and seed-stored nutrients) into the shoot tissue at the harvest date did not significantly differ between the SC and UL treatments (Table 5), whereas the UC treatment had a significantly lower total nutrient uptake in comparison with the UL treatment for these nutrients. In the case of P, the shoot tissue of the SC treatment had the highest total uptake (Table 5), whereas the SCSF treatment had the lowest nutrient uptake, although these values were not significantly different in comparison with the other treatments. DRIS indices (Table 3) highlighted differences in the supply of nutrients to the shoot for the different treatments and thus also highlighted differences in compensatory response. High values indicate high supply and low values indicate low supply for the particular nutrient. The DRIS indices for N, K and Mg were smallest for the UC treatment, for example, whereas the SC showed a compensatory DRIS balance for these nutrients.

In the soil, we found relatively high nutrient concentrations in the C(SCSF) compartment in the upper third of the rhizotron only. In all other compartments, a downward gradient was observed (Table 6) for the mobile nutrients NO3 and K, with lower nutrient concentrations in the upper third and higher nutrient concentrations in the lower third of the compartments. Our aim of doubling the nutrient concentration and the nutrient supply in the C(SCSF) compartment was probably not fully achieved by applying a double volume of nutrient solution to C(SCSF) (Table 6). Indeed, at harvest, the concentrations in C(SCSF) were not twice as high compared with, for example, C(SC). However, the
concentrations of NO₃⁻, NH₄⁺, P and K increased in C(SCSF) compared with all other compartments. In this respect, it has to be considered that Table 6 shows nutrient concentrations at harvest when the roots have already taken up nutrients. Probably, the nutrient concentration under C(SCSF) was temporarily even higher. Moreover, we aimed to establish a contrasting treatment in SCSF, which means that the nutrient concentration in L(SCSF) was intended to be low. However, this was not achieved, since the nutrient concentration in L(SC) was comparable to other compartments, probably due to mineralisation processes that occurred in the peat. Nevertheless, when SCSF was compared with SC, we conclude that a contrasting nutrient supply was offered to plants by the loose and compacted compartments.

The spatial distribution of nutrient concentration correlated with the lateral root initiation in C(SCSF), which shifted more to the upper part of the C(SCSF) compartment in comparison with the compartments from the other treatments (Table 2).

**Table 3.** Effect of localized soil compaction and fertilization on DRIS-indices (obtained from nutrient concentrations of shoot tissue), at 21 DAT. Different characters next to values indicate statistically significant differences (P < 0.05; one way ANOVA; Post-hoc test: LSD-method; arithmetic means and SE (in brackets), n=5).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uniform loose (UL)</th>
<th>Uniform compacted (UC)</th>
<th>Split compaction (SC)</th>
<th>Split compaction &amp; split fertilization (SCSF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRIS-index Nitrogen</td>
<td>-1.2 (±3.00)</td>
<td>-8.6 (±3.66)</td>
<td>-4.0 (±2.21)</td>
<td>-2.7 (±2.97)</td>
</tr>
<tr>
<td>DRIS-index Phosphorus</td>
<td>-0.4 (±3.92) b</td>
<td>15.3 (±3.24) a</td>
<td>8.5 (±2.32) ab</td>
<td>1.1 (±7.46) ab</td>
</tr>
<tr>
<td>DRIS-index Potassium</td>
<td>-3.1 (±4.70)</td>
<td>-14.4 (±0.97)</td>
<td>-5.4 (±3.22)</td>
<td>-10.9 (±4.40)</td>
</tr>
<tr>
<td>DRIS-index Calcium</td>
<td>2.2 (±2.80)</td>
<td>4.4 (±2.98)</td>
<td>-1.3 (±2.48)</td>
<td>8.7 (±4.35)</td>
</tr>
<tr>
<td>DRIS-index Sulphur</td>
<td>4.7 (±4.71)</td>
<td>6.3 (±1.86)</td>
<td>2.4 (±3.62)</td>
<td>1.6 (±5.58)</td>
</tr>
<tr>
<td>DRIS-index Magnesium</td>
<td>-2.2 (±1.60)</td>
<td>-3.1 (±1.54)</td>
<td>-0.2 (±0.60)</td>
<td>2.3 (±2.70)</td>
</tr>
</tbody>
</table>
Table 4. Effect of localized soil compaction and fertilization on nutrient concentrations of shoot tissue, at 21 DAT. Different characters next to values indicate statistically significant differences ($P < 0.05$; one way ANOVA; Post-hoc test: LSD-method; arithmetic means and SE (in brackets), n=5).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uniform loose (UL)</th>
<th>Uniform compacted (UC)</th>
<th>Split compaction (SC)</th>
<th>Split compaction &amp; split fertilization (SCSF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (%)</td>
<td>6.0 (±0.19)</td>
<td>5.7 (±0.22)</td>
<td>5.8 (±0.14)</td>
<td>6.0 (±0.08)</td>
</tr>
<tr>
<td>Phosphorus (g kg⁻¹)</td>
<td>4.7 (±0.38)</td>
<td>6.1 (±0.41)</td>
<td>5.7 (±0.25)</td>
<td>5.1 (±0.75)</td>
</tr>
<tr>
<td>Potassium (g kg⁻¹)</td>
<td>62 (±2.0) a</td>
<td>56 (±1.0) b</td>
<td>61 (±1.4) ab</td>
<td>58 (±1.3) ab</td>
</tr>
<tr>
<td>Sodium (g kg⁻¹)</td>
<td>2.3 (±0.12) a</td>
<td>1.5 (±0.18) b</td>
<td>2.1 (±0.12) a</td>
<td>2.0 (±0.15) a</td>
</tr>
<tr>
<td>Magnesium (g kg⁻¹)</td>
<td>4 (±0.2)</td>
<td>4 (±0.0)</td>
<td>4 (±0.0)</td>
<td>4 (±0.1)</td>
</tr>
<tr>
<td>Calcium (g kg⁻¹)</td>
<td>6.4 (±0.44) ab</td>
<td>6.9 (±0.19) ab</td>
<td>6.1 (±0.24) b</td>
<td>7.0 (±0.23) a</td>
</tr>
<tr>
<td>Sulphur (g kg⁻¹)</td>
<td>8.7 (±0.77)</td>
<td>8.9 (±0.37)</td>
<td>8.1 (±0.47)</td>
<td>8.8 (±0.82)</td>
</tr>
<tr>
<td>Carbon (%)</td>
<td>40 (±0.2)</td>
<td>40 (±0.5)</td>
<td>40 (±0.4)</td>
<td>41 (±0.5)</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>6.7 (±0.17)</td>
<td>7.1 (±0.22)</td>
<td>6.9 (±0.16)</td>
<td>6.8 (±0.09)</td>
</tr>
<tr>
<td>Manganese (g kg⁻¹)</td>
<td>0.3 (±0.02)</td>
<td>0.3 (±0.01)</td>
<td>0.3 (±0.01)</td>
<td>0.4 (±0.01)</td>
</tr>
</tbody>
</table>
Table 5. Effect of localized soil compaction and fertilization on total nutrient uptake (sum of uptake and seed stored nutrients) into the shoot tissue, at 21 DAT. Different characters next to values indicate statistically significant differences ($P < 0.05$; one way ANOVA; Post-hoc test: LSD-method; arithmetic means and SE (in brackets), n=5).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Uniform loose (UL)</th>
<th>Uniform compacted (UC)</th>
<th>Split compaction (SC)</th>
<th>Split compaction &amp; split fertilization (SCSF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen (mg)</td>
<td>9.7 (±0.53) a</td>
<td>7.8 (±0.45) b</td>
<td>9.1 (±0.56) ab</td>
<td>8.1 (±0.55) ab</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>0.8 (±0.06)</td>
<td>0.8 (±0.03)</td>
<td>0.9 (±0.06)</td>
<td>0.7 (±0.10)</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>10.0 (±0.43) a</td>
<td>7.6 (±0.35) b</td>
<td>9.5 (±0.64) a</td>
<td>7.8 (±0.42) b</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>0.4 (±0.02) a</td>
<td>0.2 (±0.04) c</td>
<td>0.3 (±0.03) ab</td>
<td>0.3 (±0.02) bc</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>0.64 (±0.035)</td>
<td>0.56 (±0.035)</td>
<td>0.64 (±0.032)</td>
<td>0.58 (±0.037)</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1 (±0.1)</td>
<td>1 (±0.1)</td>
<td>1 (±0.0)</td>
<td>1 (±0.1)</td>
</tr>
<tr>
<td>Sulphur (mg)</td>
<td>1.4 (±0.12)</td>
<td>1.2 (±0.03)</td>
<td>1.3 (±0.04)</td>
<td>1.2 (±0.15)</td>
</tr>
<tr>
<td>Carbon (mg)</td>
<td>64 (±2.0) a</td>
<td>55 (±2.6) b</td>
<td>62 (±2.9) ab</td>
<td>55 (±3.6) b</td>
</tr>
<tr>
<td>Manganese (mg)</td>
<td>0.05 (±0.003)</td>
<td>0.05 (±0.003)</td>
<td>0.04 (±0.001)</td>
<td>0.05 (±0.003)</td>
</tr>
</tbody>
</table>
Table 6. Effect of localized soil compaction and fertilization on nutrient concentrations in three depth levels within the rhizotrons: upper third (0-26 cm), middle third (27-53 cm), lower third (54-80 cm) of depth below soil surface, at 21 DAT. Arithmetic means and SE (in brackets); n=3; 'bdl' = below detection limit, 'nd' = not defined.

<table>
<thead>
<tr>
<th>Compart-</th>
<th>Depth</th>
<th>pH</th>
<th>K</th>
<th>Mg</th>
<th>Mn</th>
<th>P</th>
<th>NO3</th>
<th>NH4</th>
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<tr>
<td>ment</td>
<td>level</td>
<td></td>
<td>mg kg⁻¹</td>
<td>mg kg⁻¹</td>
<td>mg kg⁻¹</td>
<td>mg kg⁻¹</td>
<td>mg kg⁻¹</td>
<td>mg kg⁻¹</td>
</tr>
<tr>
<td>UL</td>
<td>upper</td>
<td>5.0 (±0.41)</td>
<td>48 (±1.8)</td>
<td>549 (±11.8)</td>
<td>13 (±0.4)</td>
<td>bdl</td>
<td>50 (±9.7)</td>
<td>3.0 (±0.35)</td>
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<tr>
<td></td>
<td>middle</td>
<td>4.6 (±0.08)</td>
<td>59 (±3.0)</td>
<td>582 (±13.8)</td>
<td>14 (±0.5)</td>
<td>bdl</td>
<td>117 (±52.6)</td>
<td>3.0 (±0.48)</td>
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<tr>
<td></td>
<td>lower</td>
<td>4.8 (±0.27)</td>
<td>65 (±2.2)</td>
<td>574 (±15.0)</td>
<td>13 (±0.6)</td>
<td>bdl</td>
<td>278 (±38.0)</td>
<td>3.4 (±0.42)</td>
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<tr>
<td>UC</td>
<td>upper</td>
<td>4.5 (±0.08)</td>
<td>54 (±1.2)</td>
<td>574 (±12.4)</td>
<td>13 (±0.2)</td>
<td>bdl</td>
<td>62 (±19.7)</td>
<td>4.7 (±0.90)</td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td>4.5 (±0.05)</td>
<td>64 (±2.2)</td>
<td>578 (±12.3)</td>
<td>13 (±0.3)</td>
<td>bdl</td>
<td>133 (±31.2)</td>
<td>3.8 (±0.40)</td>
</tr>
<tr>
<td></td>
<td>lower</td>
<td>4.5 (±0.09)</td>
<td>61 (±3.4)</td>
<td>533 (±28.4)</td>
<td>12 (±0.7)</td>
<td>bdl</td>
<td>200 (±47.3)</td>
<td>3.9 (±0.88)</td>
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<td>48 (±1.6)</td>
<td>568 (±7.4)</td>
<td>13 (±0.2)</td>
<td>bdl</td>
<td>51 (±9.2)</td>
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<td>middle</td>
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<td>566 (±9.5)</td>
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<td>bdl</td>
<td>122 (nd)</td>
<td>6.3 (±1.62)</td>
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<tr>
<td></td>
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<td>62 (±1.9)</td>
<td>574 (±21.9)</td>
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<tr>
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<td>566 (±4.6)</td>
<td>13 (±1.0)</td>
<td>3.8</td>
<td>33 (nd)</td>
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<td>595 (±18.2)</td>
<td>13 (±0.9)</td>
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<td>552 (±22.0)</td>
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<td>599 (±15.3)</td>
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<td>bdl</td>
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</table>
Figure 5. Effect of localised soil compaction and fertilisation on the dynamics of the ratio between visible root length and projected leaf area. Treatments are labelled with the abbreviations UL (uniform loose), UC (uniform compacted), SC (split compaction) and SCSF (split compaction and split fertilisation). Different characters indicate statistically significant differences ($P < 0.05$; linear mixed models; post hoc test: LSD method; arithmetic means ± s.e.; $n = 5$).
Figure 6. Effect of localised soil compaction and fertilisation on the dynamics of (a) rooting depth and (b) visible root length density (RLD) over depth in rhizotrons at 20 days after transplantation. Treatments are labelled with the abbreviations UL (uniform loose), UC (uniform compacted), SC (split compaction) and SCSF (split compaction and split fertilisation). In (a), at 20 DAT, all roots from the L(SC) compartment had reached the bottom of the rhizotrons and grew horizontally. This prevented the accurate measurement of rooting depth for L(SC) at 20 DAT. The first letter before parentheses refers to compaction within each treatment (e.g. L(SC) denotes the loose compartment within the SC treatment). All values show the mean of one compartment. Different characters indicate statistically significant differences ($P < 0.05$; linear mixed models, post hoc test: LSD method; arithmetic means ± s.e.; $n = 5$).
As an indicator for the overall physiological performance of plants grown under different localised soil compaction and applications of fertiliser, gas exchange parameters were quantified and correlated with growth responses. Gas exchange measurements were conducted after irrigation at 0900 hours. Our expectation that soil compaction generally leads to lower stomatal conductance could not be supported by the data (Fig. 8b). Testing gas exchange parameters (photosynthetic assimilation and stomatal conductance) over the course of time by two-way ANOVA (the independent factors were time of day and treatment) revealed no significant differences between the treatments. However, the factor ‘time of day’ had a significant influence on the variation of the data ($P < 0.0001$). Climate
conditions such as temperature and air humidity were highly stable at the day of gas exchange measurements (17 DAT: air temperature, 21°C ± 0.5°C; relative air humidity, 65% ± 10%). All measured gas exchange parameters of the plants, particularly the plants for which roots were grown entirely or partly in compacted soil (UC, SC and SCSF treatments), showed a minimum at 1200 hours, a local maximum in the afternoon and a decrease in the evening (Fig. 8a, b). In contrast, plants grown in loose soil (the UL treatment) did not show such a pronounced diurnal variation in the afternoon compared with the other plants. Due to the different duration of the gas exchange monitoring for the treatments (for example, for SCSF, we missed the morning and part of the evening) and due to the limited number of replicates (no reproduction on another day), these data have to be interpreted carefully and with limited statistical stringency.

![Figure 8](image_url)

**Figure 8.** Effect of localised soil compaction and fertilisation on (a) photosynthetic assimilation and (b) stomatal conductance at 16 DAT. Treatments are labelled with the abbreviations UL (uniform loose), UC (uniform compacted), SC (split compaction) and SCSF (split compaction and split fertilisation). Symbols represent arithmetic means ± s.e. from 10 repeated-measurements per point in time; n = 5.
3.5 Discussion

Our results provide insight into compensatory root growth adjustments for the model plant spring barley in response to heterogeneous soil compaction and nutrient availability. Question (a), which addresses whether it is possible to observe growth responses to heterogeneous soil mechanical strength under controlled conditions, could be answered positively. In our experiment, we found effects of soil compaction that were similar to those previously reported for controlled environment and field for maize and ryegrass (Shierlaw and Alston 1984; Konôpka et al. 2009). In particular, shoot (Fig. 1a) and root growth was drastically reduced in compacted peat (Fig. 1b) and roots were, on average, thicker in peat with a relatively high mechanical strength (Fig. 7a).

Moreover, we could observe that compensatory growth effects occurred (Question (b)). Plants for which only one side of the root system was placed in compacted soil (the SC treatment) showed compensatory effects for shoot DW production (Fig. 2a), leaf area at 20 DAT (Fig. 3a) and the number of leaves (Fig. 3b, Table 1) when equal amounts of fertiliser were applied to both sides of the root system. However, with reference to Question (c), when only the compacted compartment was given fertiliser, there were fewer roots that had access to nutrients compared with the SC treatment (Figs 2b, 3c, f). As a consequence, plants from the SCSF treatment should display growth reduction compared with the other split-root treatment, SC. Our experiments clearly showed that this is the case because plants from SCSF had reduced shoot growth (Figs 2a, 3a, b; Table 1) compared with the SC treatment. However, we observed an increased lateral root initiation and a higher proportion of lateral root length in the exclusively fertilised compacted compartment than in the compartment without fertiliser (Fig. 4c). Also, as a trend, C(SCSF) resulted in a higher lateral root number in the upper part of the rhizotron compared with C(SC) (Table 2). These findings underline the importance of unimpeded root growth for an appropriate acquisition of mineral nutrients from the rhizosphere.

Effect of soil physical conditions

Severe soil compaction, together with excess soil water, can reduce the pore volume that is filled with air (the air-filled porosity). Commonly, air-filled porosity below 10–12% is considered to reduce the soil’s permeability to oxygen and result in root growth inhibition
In our experiment, air-filled porosity within the compacted soil was greater than 14%. Therefore, it is unlikely that oxygen deficiency might have occurred in compacted soil and we conclude that the observed effects for the different treatments are mainly a result of the varying mechanical resistance of the substrate. In these treatments, soil compaction increased mechanical resistance (penetrometer resistance) 22-fold in comparison with loose soil, whereas soil bulk density differed by only 1.6-fold. This is expected, because in soils, there is usually no linear relationship between bulk density and mechanical resistance. Moreover, it has to be considered that the chosen peat substrate has an extremely low oven-dried density in comparison to a typical field soil.

**Effect of homogeneous soil compaction**

Numerous studies highlight that uniform soil compaction leads to a substantial reduction of both root and shoot growth, and to delayed plant development (Montagu *et al.* 2001; Lipiec *et al.* 2003). Decreasing lateral root length and leaf number has been reported for maize and triticale (× *Triticosecale* Wittmack; wheat–rye hybrid) affected by soil compaction (Grzesiak 2009). A reduced number of leaves was observed also for yam (*Dioscorea alata* L.) and tomato (*Solanum lycopersicum* L.) plants growing in compacted soil (Ferguson and Gumbs 1976; Mulholland *et al.* 1999). In the C₄ bunchgrass *Schizachyrium scoparium* (Michx.) Nash, a decrease in tillering was observed when soil around plants was compacted severely (Wallace 1987). In line with previous findings, our results show that the UC treatment resulted in slower plant development compared with the UL treatment. These effects were brought about by reduced leaf appearance (Fig. 3b), leaf area (Fig. 3a) and newly formed lateral roots (Fig. 3e), which showed a tendency to be correlated in the UL and UC treatments.

These findings support the view that the experimental system used here was suitable for studying simultaneously integrated shoot and root responses to uniformly compacted soil. Interestingly, these responses were evident even when the choice of substrate, the age of the plants, the duration of the experiment and the dimensions of the rhizotrons differed strongly from the experimental setup in previous studies.
Effect of heterogeneous soil compaction in the SC treatment

Regarding shoot biomass (Fig. 2a), leaf number (Fig. 3b), leaf area (Fig. 3a) and root : shoot ratio at 20 DAT (Table 1), the plants from the SC treatment were able to compensate in comparison with plants from the UL treatment. The same trend was observed for the number of tillers \((P = 0.09; \text{ Table 1})\). These effects were accompanied by several changes in the RSA parameters and root development in the loose L(SC) compartment, specifically rooting depth (Fig. 6a), total root length (Fig. 3c, f), earlier occurrence of laterals (Fig. 4), root surface area and overall shape estimated by the convex hull (Table 2).

The response of wheat \((\text{Triticum aestivum L.})\) and barley roots to vertically split heterogeneous soil compaction was studied earlier by Bingham and Bengough (2003). In their study, the experimental system was different and plants were analysed only at one time point (12 DAT). Bingham and Bengough used cylinders in which the root zone of barley plants was divided into a compartment filled with densely compacted soil and a compartment with loose soil. The effects on leaf area that we report here (Fig. 3a) confirm the results of Bingham and Bengough (2003), as the leaf area from the plants grown in uniformly compacted soil was significantly smaller than that of shoots grown both in the SC treatment and in the UL treatment. Likewise, the increase in lateral root initiation in the loose compartment of the split-root treatment compared with uniform treatments (Fig. 4, Table 2) was also found by Bingham and Bengough (2003). In addition, by quantifying root growth via imaging, we could show that the effect of localised soil compaction on RSA traits increases over time, and different treatments resulted in different dynamics of lateral root initiation. At 12 DAT (the harvesting time of Bingham and Bengough 2003), the treatment effect on the ratio between lateral and seminal root length had just become apparent and continued to increase until the end of our observations (20 DAT, Fig. 4).

Effect of heterogeneous soil compaction and fertilisation in the SCSF treatment

While the SC treatment received the same amount of fertiliser as the uniform treatments, with the fertiliser divided on both compartments of the split-root rhizotrons, the SCSF treatment received fertiliser solely via the compacted compartment. Plants from the SCSF treatment were not able to compensate in terms of shoot DW (Fig. 2a) and the number of leaves (Table 1) in comparison with UL and SC, and developed a similar number of tillers to those in the UC treatment (Table 1).
The proportions of seminal + nodal and lateral root lengths were significantly different for plants grown in the SC and SCSF treatments (Fig. 7b, c). Regarding the proportions of seminal and lateral root length, C(SC) was comparable to UC, and L(SC) was comparable to UL. On the other hand, C(SCSF) resulted in a significantly higher proportion of lateral roots than UC and C(SC). These results show that the plants from the SCSF treatment invested locally in the C(SCSF) compartment in lateral root growth, triggered by the reduced nutrient availability. Furthermore, in the upper third of the C(SCSF) compartment, a greater number of lateral roots (Table 2) was found in comparison with all other compartments, except for UL. Moreover, as a trend, the root surface area of the L(SCSF) compartment was smaller than that of L(SC), and the root surface area of C(SCSF) was smaller than that of C(SC) (Table 2). Also, the convex hull for L(SC) showed a trend to be higher in comparison with UL (Table 2). All these differences imply that a different strategy for nutrient acquisition was adopted by roots exposed to these two split-root treatments.

Root growth of plants from the uniform UL and UC treatments was in line with shoot growth, because the reduced shoot growth (Figs 2a, 3a) and development (Table 1, Fig. 3b) of plants from the UC treatment can be explained by the restricted root growth in UC (Figs 2b, 3c–f). Similarly, plants from the SC treatment showed compensatory root growth as described above, which was sufficient to maintain the growth of the shoot (Fig. 2a). However, plants from the SCSF treatment also showed compensatory root growth (although this was different from that in the treatment SC), especially in lateral root initiation in the C(SCSF) compartment (Figs 4, 7c; Table 2). This compensatory growth was not sufficient for maintaining shoot growth compared with plants from the UL and SC treatments (Fig. 2a). The different partitioning into the root orders (seminal and nodal vs. lateral roots), the different depth of growth and a slightly different root length between plants from SC and SCSF cannot explain why plants from the SC treatment were able to compensate and plants from the SCSF treatment failed to compensate regarding shoot growth and development (Figs 2a, 3a, b; Table 1). The reason for this different ability, to respond to localised compaction was the different access to nutrients of the two split-root treatments. Why plants from SCSF failed to compensate can only be answered when nutrient concentrations in the soil and nutrient uptake are taken into account.

Limed black peat can be considered a poor substrate for several nutrients, including P, N and K. Due to this reason, the nutrient availability in L(SCSF), which did not receive fertiliser, can be assumed to be insufficient to promote fast growth for an extended period.
of time. Nutrient concentration in the shoot tissue (Table 4) and the total nutrient uptake into the shoot tissue (Table 5) show that the plants from SCSF were less optimally supplied with nutrients when compared with SC and UL, although the compacted side in SCSF received fertiliser twice. Shoots from the UC treatment showed a trend towards a higher P and Ca concentration than those under UL (Table 4). Since the shoot biomass in UC was reduced compared with UL, this led to a comparable total uptake for these elements into the shoot tissue for UL and UC (Table 5). However, the total nutrient uptake of K and N in the shoot tissue of plants from the UC treatment was significantly reduced compared with those under the UL treatment. This shows that for nutrients such as K and N, which are more mobile than P and Ca, plants growing in moderately compacted peat are at a disadvantage compared with plants growing in loose peat.

Total nutrient uptake could be compensated for K in SC compared with plants from UL, whereas plants from SCSF showed a lower K uptake into the shoot tissue than plants from SC. Similar trends were present for N, P and S. In compacted soil, P uptake per unit root length can be increased in comparison with loose soil, as reported for ryegrass (Shierlaw and Alston 1984), probably as a consequence of the low mobility of P and greater contact area between soil and root due to compaction. The development of rooting depth in the different compartments of the treatments in the course of time indicates another different compensatory strategy, which varied with the treatments (Fig. 6a). Deep root growth might be advantageous for the nutrient uptake of the L(SC) treatment because the highest nutrient concentrations were found in the lower third of the rhizotrons (Table 6). This downward gradient might be created by both nutrient uptake and leaching. For C(SCSF), it might be less advantageous to grow deep because the nutrient concentration was comparatively high in the upper third of the C(SCSF) compartment. The reason here might be the double amount of fertiliser given to C(SCSF) at 0, 7 and 14 DAT, and generally slower leaching from compacted soil. Therefore, in case of C(SCSF), the possible foraging for nutrients by the development of deeper roots was not as pronounced in comparison with C(SC), where the nutrient concentration substantially increased following a downward gradient (Table 6). Another observation might support the finding of reduced depth growth in C(SCSF). In the lower third of the C(SCSF) compartment, not a single root was found when roots were washed out of the soil, in contrast to all other compartments, where always at least a few roots were found. Altogether, nutrient availability and growth in depth were consistent. The spatial distribution of nutrients shown in Table 6 correlates with root morphological adjustments regarding lateral root
initiation (Table 2; Figs 4, 7), especially for C(SCSF), and also root growth dynamics, especially regarding the depth growth (Fig. 6a) and lateral root growth (Fig. 4) of L(SC).

Dynamic development of lateral roots

Our results clearly show that lateral root initiation was accelerated in the loose compartments of the split-root treatments in contrast to the other compartment of the same rhizotrons filled with compacted soil (Fig. 3e). This demonstrates an important plant-internal feedback mechanism between different portions of the root system, which might be mediated by a probabilistic regulation via plant hormones such as auxin. Plants are able to induce new laterals within the root system at soil locations with favourable growth conditions, particularly concerning nutrients such as P and N (Laskowski 2013). Our data imply that a small time window between Day 13 and 20 after the onset of treatments was decisive for the establishment of the observed responses (see Fig. 4). This time window might be species-specific. In tomato exposed to uniform soil compaction, an increased number of lateral roots were already found at 10 days after the onset of the treatment when loose and compacted soils were compared (Tracy et al. 2012). Soil compaction frequently influences the ratio of lateral to seminal root length (Goss 1977; Bingham and Bengough 2003). Lateral roots are sometimes still able to penetrate the thin pores remaining in compacted soil, whereas seminal roots are already impeded (Shierlaw and Alston 1984; Bingham and Bengough 2003). In the present experiment, these results could not be confirmed in general (e.g. not for UC (Figs 3e, 4a, Table 2)), where plants did not show an increase in lateral root growth. However, roots in compacted soil were indeed able to increase lateral root growth, as observed for C(SCSF) (Figs 3e, 4c). Lateral root number (Table 2) and the length proportion of second order laterals were, as a trend, increased in C(SCSF) (Fig. 7c), the twice-fertilised compacted compartment of treatment SCSF, compared with UC and C(SC). This can be interpreted as an optimisation strategy for nutrient uptake. Applying fertiliser to the compacted compartment only, like in the SCSF treatment, is certainly less effective for the nutrient supply of the plant in comparison with the fertilisation regime applied in all other treatments. The development of the ratio between visible root length and leaf area (Fig. 5) in the SC and SCSF treatments, where only part of the root system was growing in compacted substrate, was intermediate between the UL and UC treatments. This result shows that compensation with respect to root growth was not related to an increase in overall root
length. The compensatory effects with respect to root growth in the SC and SCSF treatments were a result of alterations in RSA, root morphology, and the dynamic development of rooting depth and lateral root initiation.

Gas exchange as an indicator for the time of the day of maximal stress

The two split-root treatments showed different acclimation strategies with respect to root growth. Moreover, plants from the SCSF treatment were not able to maintain shoot growth to a rate as high as of those subjected to the SC treatment. In a subsequent step, we asked if the treatments were accompanied by further physiological differences, particularly with regard to water loss by transpiration and photosynthetic rates. Soil compaction is known to affect the balance of the plant hormone ABA (Tardieu et al. 1991; Mulholland et al. 1996a; Mulholland 1996b). Due to this reason, we tested the physiological effect of the treatments on gas exchange parameters. We expected that plants from the UC treatment would have the lowest stomatal conductance and that the plants from the split-root SC and SCSF treatments would possibly have a lower stomatal conductance than UL because plants from these treatments were confronted partly with compacted soil. However, these expectations could not be confirmed by our results (Fig. 8). Rather, we observed diurnal variations in the gas exchange parameters (Fig. 8a, b). At 1200 hours, the rhizosphere in the compacted compartments was probably more deprived of water due to water uptake by the roots than those in loose compartments because the hydraulic conductivity of peat soils is drastically reduced after compression (Hamza and Anderson 2005; Wong et al. 2009). For a specific soil water content, the hydraulic conductivity depends on the pore size distribution of the soil and consequently on compaction (Bear and Veirujt 1987).

The observed diurnal variations in the transpiration rate (especially visible for UC) were probably due to this relative drying of the rhizosphere as a consequence of the combination of water uptake and subsequent reduced delivery of water resulting in a lower (more negative) water potential. The more negative the matric potential of the soil, the lower the transpiration rate (Denmead and Shaw 1962). This possible shortage of water supply might have increased the concentration of ABA, resulting in a decrease in the stomatal conductance at 1200 hours for all treatments containing compacted soil (Fig. 8b). This scenario would also explain why the range of stomatal conductance was greater for
the treatment SCSF than that for SC (Fig. 8b). Water uptake from C(SCSF) was probably greater than that from C(SC) due to the trend of increased number (Table 2) and length proportion (Fig. 7c) of lateral roots in the upper part of the rhizotron and subsequently root length density until a depth of ~30 cm (Fig. 6b).

Another possible explanation for the reduction of the transpiration rate at 1200 hours was the possibly higher soil strength in consequence of the relative drying of the soil. Dodd et al. (2010) observed that the penetrometer resistance decreases with increasing water content in all soils and also in peat. Probably, it was a combination of both the increase in soil strength and lower water potential. Both increase in soil strength and water content, can result in decreases of stomatal conductance and leaf expansion (Masle and Passioura 1987). The increase in the transpiration rate in the afternoon can be explained by a more negative leaf water potential, which typically increases over a day (Slatyer 1967).

In future studies, it would be interesting to analyse the response of root and shoot growth to soil compaction during different times of the day.

3.6 Conclusion

Our study demonstrates that spring barley shows substantial phenotypic plasticity for lateral root emergence and maximal rooting depth when growing in heterogeneously compacted and fertilised substrate. We show that under these unfavourable conditions, global RSA and shoot responses occur in a timeframe of a few days after the onset of treatments, resulting in compensatory effects and acclimation. These observations will assist the design of future studies to elucidate the physiological mechanisms of these growth responses to soil physical conditions, and to analyse genotypic diversity in barley and other plant species.

3.7 Acknowledgements

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Chapter 4

Evaluation of electrical impedance tomography (EIT) for the characterization of root growth and function

4.1 Abstract

A better understanding of soil-root interactions is essential to achieve progress in crop breeding, crop management and the calibration of rhizosphere models. To date it is still difficult to analyze root growth and function in the field because of a lack of satisfactory non-destructive monitoring methods. A promising technique in this respect is electrical impedance tomography (EIT), which utilizes electrical conduction and polarization properties of the soil-root system in an imaging framework. We investigated the capability of EIT in the laboratory to image root growth in soil-filled rhizotrons (78 × 30 × 2 cm) by comparing the electrical imaging results with those obtained from established optical methods. A series of experiments were conducted using the monocotyledonous crops barley and wheat and the dicotyledonous crop oilseed rape as model plants. In several experiments, daily EIT measurements were conducted over several weeks and root growth was monitored using the image analysis system GROWSCREEN-Root. Correlations between EIT imaging data and root growth parameters were analyzed. We intended to answer the question to what extent different root system morphologies and soil properties (water content, ion concentration, macropores) affect the electrical impedance spectra of soil-root systems. We did not observe differences between plants monitored over several weeks with EIT and control plants, indicating that the measurement process itself does not affect plant performance. Our results show that EIT is applicable, under controlled conditions, to monitor processes related to root growth and function in the soil. However, direct characterization of root size and root growth is not possible yet and will likely remain very difficult under conditions close to field cultivation.
4.2 Introduction

Methods that would allow root growth monitoring under field conditions would be of great benefit for basic and applied research (Pierret et al. 2005, Luster et al. 2009, Fiorani and Schurr 2013, York et al. 2013). Immanently, such methods would have to be non-destructive and non-invasive. This means that the natural soil-root system under investigation may not be invaded or disturbed by the measurement device, for example by digging or inserting the measurement device into the soil. Furthermore, all physiological processes in the plant, such as growth, development and uptake of water and nutrients, may not be influenced by the measurement. Such a method does not exist to date. Until now, this unsolved challenge and the resulting methodological limitations have led to in situ studies of root growth being to a large extent neglected and widely treated as a black box in practical considerations on the field scale (Silberbush 2013).

A considerable number of studies highlight the importance of crop roots and their size, depth of growth and root system architecture (RSA) for the uptake of water and nutrients (Lynch 1995, Hinsinger et al. 2009, Hodge et al. 2009, Gaiser et al. 2012). The root functions of water and nutrient uptake are pivotal for yield formation. Many essential advances in the area of non-invasive root growth monitoring in soil were made in the last decade on the laboratory scale, especially regarding improvement of spatial and temporal resolution. The results obtained with these new methods have deepened the understanding of many processes in the rhizosphere that are essential for the acquisition of nutrients (Nichol and Silk 2001). For example, X-ray computed tomography (Tracy et al. 2010, Mairhofer et al. 2012), NMR/MRI (Nagel et al. 2009; Jahnke et al. 2009), PET (Jahnke et al. 2009), and optical growth monitoring in rhizotrons with transparent walls (Nagel et al. 2012) all allow for the characterization of RSA traits and root growth. In the field, several optical methods allow for quantification of RSA and root growth along profile walls of excavations (for a review see Gaiser et al. 2012) or along transparent walls of perspex tubes ('mini-rhizotrons') that are inserted in the soil (for a review see Smit et al. 2000, Polomski and Kuhn 2002). Yet, these methods require alteration of the rhizosphere in order to allow for direct observation of roots in a two-dimensional interface of the soil. Moreover, it is impossible to visualize the entire, three-dimensional root system with these methods.
In recent years, a remarkable number of studies have used geoelectrical imaging methods for quantification and indirect imaging of root systems in soil. Particularly, electrical resistivity tomography (ERT) has proven useful for the characterization of water content in soil, which has been correlated with water uptake by roots (Michot et al. 2003, al Hagrey 2007; Srayeddin and Doussan 2009). ERT is based on the measurement principle that water content and electrical resistivity of a certain location within the soil are negatively correlated: the higher the water content, the lower the electrical resistivity of the soil element. Using such ERT-approaches, the coarse distribution of roots within a massive tree-root system could be resolved (Amato et al. 2008). A less clear result was achieved with ERT for characterization of root mass density of the herbaceous plant alfalfa in the field (Amato et al. 2009). There, the electrical resistivity signal of soil containing roots was of the same order of magnitude as the effects of soil parameters such as grain size and water content. Consequently, it was very difficult to distinguish between the root system and the surrounding soil.

In the 1970s, an alternative approach based on analyses of electromagnetic properties of the soil-root continuum was tested for the first time: Chloupek (1972, 1977) applied electrical impedance spectroscopy (EIS), a method that is also called spectral induced polarization (SIP), for the estimation of root mass in the soil. The term electrical polarization means in this context the strength of a spatial charge separation, e.g. on surfaces of roots and in the rhizosphere or soil. Capacitance is a measure of the separation of electrical charges and it depends on the capability of interfaces to carry electrical charges without allowing an electrical current to flow and diminish the separation of these charges. A critical component for the analysis of capacitance is how rapidly charges are separating in a given voltage difference between electrodes. Therefore, in practice, alternating currents are typically used to determine the capacitance of a system. In his experiments Chloupek (1977) plugged one electrode into the petiole of a carrot plant (among other species) and another electrode into the soil at a distance of 20 cm from the plant and determined the capacitance between the electrodes. Chloupek (1977) was able to demonstrate that overall root mass was related to the capacitance of the analyzed soil-root system using a rather small number of frequencies between 0.1 and 10 kHz. Dalton (1995) developed a concept of the model explaining the root capacitance discovered by Chloupek (1972). It is unclear whether the chargeability of roots, which consist of polarizable membranes, dominates the electrical properties of the soil-root-continuum or
whether factors such as soil pore geometry, water or mineral nutrient content superimpose the root signal to an extent that makes root determination impossible. Therefore, we conducted a series of experiments to determine the intensity of the above-mentioned and further factors affecting the electrical properties of the soil-root system.

The aim of our study was to determine whether a combination of the spatial differentiation of ERT and the quantification of the root biomass via SIP is powerful enough to render a volumetric representation of the root biomass per volume element in a given soil region. The combination of ERT and SIP has been established before and is commonly used – at a larger spatial resolution – to quantify ore and water distributions in larger regions of the pedosphere (for a review see Kemna et al. 2004). This method is called ‘electrical impedance tomography (EIT)’ and until now had not been used to try to assess the morphology of a root system in situ.

4.3 Materials and Methods

The present work was embedded in the research group FOR 1320 ‘Crop Sequence and Nutrient Acquisition from the Subsoil’ of the DFG (German Research Foundation) and was designed for the support of root data acquisition on the field trial CeFiT (silty loam soil, Haplic luvisol, at the research station Klein-Altendorf). FOR 1320 focuses on the investigation of the importance of the subsoil and its preparation by preceding crops for the acquisition of nutrients by following crops. The first project phase intended to investigate the applicability of the EIT methodology for the characterization of root growth and function in field soil at the laboratory scale by use of rhizotrons.

The present investigations involved several experiments in which electrical properties of roots and root-surrounding media (aqueous solutions and soil) were quantified using a spectral induced polarization (SIP) system or using a 40-channel electrical impedance tomography system (EIT) called MEDUSA II. Basically, with SIP and EIT, the same quantities can be determined, but whereas SIP is usually not used to generate images of the spatial distribution of the electrical quantities in the object under investigation, EIT is an imaging technique. Both systems were described previously (Zimmermann et al. 2008, Zimmermann 2010). Roots from barley, oilseed rape and wheat were investigated and different media in which roots can grow were tested, such as aqueous solutions containing...
different concentrations of ions as well as growing substrates and soils. All experiments were either conducted in small plastic containers (1.5 cm wide, 2.1 cm deep, 16.0 cm long; allowing SIP-measurements, Fig. 1a) equipped with up to six brass electrodes, or in rhizotrons (30 cm wide, 78 cm deep, 2 cm thick; allowing EIT measurements, Fig. 1b) equipped with up to 38 electrodes. All brass electrodes used had a diameter of 6 mm.

Plant cultivation

The rhizotrons were filled as described previously (Nagel et al. 2012) by pressing the soil in layers (for each layer: 500 mL = 230 g for moist peat or 675 g for moist field soil) into the rhizotrons (4.68 L volume; pressure load for compaction: 0.1 MPa to 0.2 MPa) to prevent soil settlement with time. Plants were directly sown into the soil and grown in a controlled environment chamber (Fig. 4) at 21°C air temperature day and night with a photoperiod of 12 h / 12 h light / dark. Relative air humidity was maintained at 65%. Typically, plants were supplied with 50 ml of water every day to keep the soil water content comparable for the entire soil volume. Illumination was supplied by fluorescent tubes (Osram L 58W/77 Fluora 58 W, Osram GmbH, Munich, Germany) that provided 240 µmol m⁻² s⁻¹ photosynthetically active radiation (PAR) measured at the initial plant height. To prevent light reaching the roots and to prevent algae growth on the transparent plates of the rhizotrons, the transparent plates were thoroughly covered with black cloth except when being photographed. Details on plant cultivation and treatments will be explained in the following sections.

Analysis of root growth by optical imaging and conventional methods

Images of the root systems were acquired using a custom-made photo-box and SLR camera (Canon EOS digital 400D, 10 Mpx, with a 28 mm EFS lens) that were described previously (Nagel et al. 2012). The photo-box was equipped with a black cardboard background with a hole through which the camera lens was positioned to minimize reflections on the transparent plates of the rhizotrons. The custom-made image analysis software GROWSCREEN-Root was used to quantify root length density. At harvest, the soil of the rhizotrons was carefully cut in pieces of uniform size (typically, a grid of 5*13 pieces was used) and the soil’s fresh and dry weights were determined. Roots in these soil
pieces were washed out and root length was determined using WinRHIZO (images with 400 dpi, Regent instruments Inc., Canada). Root dry weight was determined after drying the washed roots at 70°C for three days.

**Analysis of shoot biomass and shoot development**

At harvest, shoot dry weight of the individual plants was determined after drying the shoots at 70°C for three days. Developmental parameters, number of leaves and canopy height, were determined manually.

**Analysis of the electrical conductivity of the soil solution**

1 mL of soil solution was collected once a week in the upper, middle and lower rows of the electrodes by opening all screw caps not used for electrodes and inserting microsuction cups through these holes into the soil. The soil solution was collected in Eppendorf cups by means of a vacuum pump and pooled for each electrode row. This sampling method was described previously by Göttlein *et al.* (1996) and Schreiber (2010). For the pooled samples, electrical conductivity was measured using a combined EC/pH meter.

**SIP and EIT measurements**

The principles of EIT and the exact function of the systems and algorithms used were explained previously (Zimmermann *et al.* 2008, Zimmermann 2010, Kemna 2000, Kemna *et al.* 2011, Weigand *et al.* 2011, Weigand *et al.* 2012a, Weigand *et al.* 2012b, Weigand *et al.* 2013). Here, the measurement principle will be described briefly. EIT results consist of values of the complex electrical impedance (hereafter abbreviated as “impedance”). Impedance is the apparent resistance of an alternating current. In alternating current the direction of the electron flow changes with a certain frequency. Due to the time dependency of the input there are time dependent processes in the output possible when processes of charging and storing electrical energy occur. Therefore, the impedance consists of two components: the direct current conductivity (the reciprocal is the direct current resistivity known from Ohm's law, measured in Ω), which is also called the real component (or real part) of the complex resistivity, and the imaginary component of the
resistance (the reciprocal is the imaginary conductivity, also measured in \( \Omega \)), which describes the time dependent phenomena (in consequence of the changing direction of electron flow) that are measured in the impedance (apparent resistance). The correlation between these quantities is illustrated in Fig. 2. Imaginary components of the impedance occur when there are inductors or capacitors in the electric circuit. Inductors generally do not occur in the systems that we have used for the present investigation and can thus be excluded from consideration. In our soil-root systems capacitances can be observed. Capacitors are capable of storing electrical energy. In the simplest case, capacitors are built of two conductive plates between which a layer of a low conductive substance (dielectric) is embedded. The plates of the capacitor are charged with electrons. In the impedance signal a shift in time can be observed between the maxima of current and voltage when the electric circuit containing a capacitor is set under an alternating current (Fig. 2). The current reaches its maximum earlier than the voltage. In the vector diagram this phenomenon can be quantified in terms of the phase angle (or phase angle signal) between current and voltage (Fig. 2).

Capacitances of 'root-capacitors' are the major subject of study using EIT to estimate root mass (or more precisely root surfaces) because root surfaces (inner and outer) react as capacitors. This phenomenon has been called root capacitance and was described in the last century (Chloupek 1972, Chloupek 1972) and was modeled by Dalton (1995).

In the present experiment we determined the impedance that was apparent at the electrodes attached to the objects under investigation (liquids, soils, substrates, roots). For this purpose, four electrodes were combined in a so-called geoelectrical four-point configuration (Fig. 3a). Two of these electrodes were injecting current and two of these electrodes were used for measurement of electrical potential. For EIT measurements, the same measurement principles (four-point configuration) were used, but in total 38 electrodes were partitioned into groups of four electrodes each (Fig. 3b). From the results achieved with these multiple four-point configurations, electrical tomograms were calculated using the electrical inversion algorithm CRTomo, developed by Kemna et al. (2000), and further developed by Weigand et al. (2012a, 2012b, 2013). The tomograms either show the 2D distribution of the real or imaginary part of the impedance or the 2D distribution of the magnitude (=amplitude) or phase angle within the soil-root system in the rhizotrons (Fig. 2). For EIT measurements a voltage of 9 V, a current of 100 mA, and typically 18 frequencies between 0.1 Hz and 45 kHz were used. The measurement duration depends strongly on the range of the recorded frequencies. In order to optimize
the frequencies for the detection of the root signals we recorded a huge frequency spectrum that may be narrowed down for practical applications. A single measurement lasted typically 1.5 – 3 h in our experiments, allowing for a temporal resolution of 1.5 - 3 h (8 - 16 measurements per day are possible to collect spectra). Using optimized single-frequency approaches, the temporal resolution may be improved to the range of a few minutes. The spatial resolution depends mainly on the distance of the electrodes and the inversion method. Our data were resolving typically to 4-6 cm pixel size due to the electrode distance of 4-6 cm. The reason for this rather large electrode distance is the risk of electrode polarization when the distance between the electrodes is too small.

**Statistical analysis**

In experiment 7 we investigated whether daily EIT measurements affect the growth and development of the plants. Therefore, we compared plants measured by daily EIT with plants not measured by EIT, but grown under the same conditions as plants imaged with EIT. For comparison of shoot biomass (shoot dry matter), independent-samples t-tests were conducted using the SPSS statistical software package (version 17.0, SPSS Inc., USA).

For the task of comparing and correlating our spatial data from the EIT measurements and the optical measurements we have chosen Map Comparison Kit (MCK, version 3.2.1, RIKS BV 2003) software, which has algorithms that allow for comparison of the spatial similarity of two 2D grid-maps (these are the EIT tomograms and the RLD maps). Here, for calculation of the spatial similarity of the two maps being compared, neighboring regions around single coordinates (pixels) are taken into account for the computation of the similarity (in %) of the spatial structures. Artifacts and noise in our 2D-distributed data can be handled much more powerful by calculating the spatial similarity using MCK than by cell-by-cell comparisons using simple regression analyses. For the calculation of spatial similarity in our data, a fuzzy numerical comparison algorithm with a radius of neighboring of 4 and a halving distance of 2 was chosen.

In the present investigations the spatial 2D distributions of root length density (RLD) had to be correlated with the spatial 2D distributions of the electrical impedance parameters (e.g. phase angle or imaginary component) at several points in time in order to test whether the growth of roots is quantifiable with our EIT method. The parameters of the soil-root system (RLD, water content, ion concentration) and their spatial distribution
inevitably change with time. Furthermore, these changes occur in part dependently and in part independently from each other. Moreover, periods of bad contact between an electrode and the soil cannot be excluded for the entire duration of the experiment since the soil was irrigated and probably settled. These bad contacts can lead to deviations (artifacts) in the 2D distribution of the electrical impedance parameters with the consequence of an overestimation of this electrode in the EIT image. For this reason, along with the complexity of the soil-root system under investigation, we considered using common statistical analysis methods such as regression analyses (comparing cell by cell) as inappropriate to correlate the 2D root growth data and the 2D electrical data.
Figure 1. a) Test cell with a single barley plant, filled with tap water, for SIP-measurement. b) Rhizotron with a bunch of barley plants, filled with tap water, for EIT measurement. The localization of the roots is visible through the transparent plate of the rhizotron.
Figure 2. Vector diagram of the complex impedance that projects the oscillation of the current (I, red curve) and the voltage (U, black curve) from the coordinate system into the unit circle (left side) in order to make the phase angles visible. In this example the arrows in the unit circle show the situation of an ideal capacitor after a quarter of the total period (rotation) of the current, highlighted by the vertical dashed line. The black arrow in the unit circle represents the voltage and shows 0 because the voltage is 0 V in the example, while the red arrow represents the current and shows the maximum current in this example. As an ideal capacitor is shown here, the current reaches the maximum earlier than the voltage and is ahead. This advance is measured by the phase angle (φ). The ratio between current (I) and voltage (U) is the resistance (R) (in Ω). This relation is known for the case of direct current as Ohm's law: R=U/I, and can be generalized for the case of an alternating current resistance (impedance, Z): Z=U/I, where U and I are represented as complex-valued functions of time. In the example of an ideal capacitor, the imaginary component of the resistance is negative while the real component is 0. Both the imaginary resistance as well as the real resistance can be expressed in the unit Ω.
Figure 3. a) Example for a geoelectrical four-point configuration where the current is injected via electrodes A and B, and the potential (impedance) is measured via the electrodes M and N. This measurement principle is used with more than four electrodes in electrical impedance tomography (EIT), where images are calculated by use of inversion algorithms on the basis of the single configurations. b) Principle of tomographic measurement scheme (presented in Weigand et al. 2012a,b) for current injection dipoles (C1, C2; blue arrows) and voltage measurement dipoles (P1, P2; red arrows) for the EIT measurements in rhizotrons.
4.4 Results

**Experiment 1: Impedance spectra of roots in aqueous solutions measured by SIP**

We investigated whether polarization and capacitance (phase angle and chargeability, respectively) of roots in different aqueous solutions can be measured by means of electrical impedance and how the signal changes with the applied frequency. Roots were differentiable and detectable in aqueous solutions by means of the phase angle using the SIP-method (Figs. 5a, d). In deionized water, tap water and nutrient...
solutions roots showed a higher phase angle signal than deionized water alone (Figs. 5a, d). Furthermore, using rhizotrons and EIT, a linear relation was observed between the chargeability of roots in tap water and the root dry matter of the samples that was determined afterward (Fig. 5b). This means that in this system, root biomass can be measured by EIT.

**Figure 5.** Experiment 1 investigating whether polarization and capacitance (phase angle and chargeability, respectively) of roots in different aqueous solutions can be measured by means of electrical impedance and how the signal changes with the applied frequency. a) Complex resistivity phase response of barley roots in tap water. b) Chargeability (normalized low-frequency Cole-Cole type) versus root mass of oilseed rape in tap water. c) Complex resistivity magnitude response and d) complex resistivity phase response of barley roots in different ambient solutions. Data were presented in Kemna et al. 2011.
Experiment 2: EIT on oilseed rape planted in field soil

After proving that it is possible in principle to measure root biomass with SIP, we made the step to EIT in soil-filled rhizotrons. We investigated whether it is possible to image the capacitive properties of roots growing in field soil by using EIT.

For this experiment, we dug out three winter oilseed rape plants from the field (October 2011, field trial from DFG FOR 1320 in Klein-Altendorf) and planted them into a rhizotron that was filled with topsoil from the same field. The results are shown in figure 6, which demonstrates that although the spatial distribution (2D distribution) of the magnitude (Fig. 6b) was heterogeneous and obviously rather uncorrelated to root distribution (Fig. 6a), the spatial distribution of the phase angle (Fig. 6c) was strongly correlated to the spatial distribution of the root systems of the oilseed rape plants. At the position of highest root density (in the middle of the topmost part of the soil, Fig. 6a), the phase angle signal was highest and reached a value of -35 mrad. At positions with a lower root density, the phase angle signal was lower and reached values between -15 and -25 mrad. The soil was watered from above stepwise with 500 ml of tap water over 12 hours, and the water did not infiltrate completely to the bottom of the rhizotron. This inhomogeneous distribution of soil moisture was imaged via magnitude (Fig. 6b), but did not affect phase signal (Fig. 6c) to the same extent: heterogeneities in water content (relatively dry soil at the bottom of the rhizotron and comparatively wet soil in the middle and upper part of the rhizotron) were visible in the magnitude signal (Fig. 6b), but they were hardly visible in the phase angle signal (Fig. 6c). In contrast, the spatial distribution of root length density (RLD, Fig. 6a) correlated strongly with the spatial distribution of the phase angle (Fig. 6c), while the spatial distribution of the magnitude (Fig. 6b) did not clearly reflect the spatial distribution of the root systems.

Experiment 3: Effects of pores and fertilizer solution on EIT signals

In the region where the roots were planted into the soil in experiment 2, we observed a low magnitude signal. Since air might be entrapped around the roots by the planting procedure, in the next experiment (experiment 3) we tested whether the signal might have its origin in air-filled macropores. In field soils, macropores are often generated due to bioturbation by earthworms or roots (biopores), or by mechanical processes in
consequence of freeze-thaw events or soil cultivation. In order to mimic such processes, we tested whether macropores have an effect on the complex resistivity response by generating artificial macropores in the soil that was filled in the rhizotrons. Figure 7 (experiment 3) shows the results of EIT on the oilseed rape plants that were shown already in figure 6 (experiment 2) after pricking artificial macropores into the soil. Neither 15 pores (15 mL pore volume, air-filled) pricked into the soil (not touching the transparent plate or the electrodes) nor an additional 15 pores (15 mL pore volume, air-filled) pricked along the transparent plate (the position of the pores is shown in Figure 7e) changed the four different EIT result images (magnitude (Fig. 7a), phase angle (Fig. 7b), real component of complex conductivity (Fig. 7c), and imaginary component of complex conductivity (Fig. 7d)). This result clearly shows that macropores are not a major issue for EIT on soil samples. However, when 300 ml of a fertilizer solution (50% Hoagland solution, 1075 µS cm⁻¹) were infiltrated into the pores, a strong decrease in the magnitude resistance and a strong increase in the real component of complex conductivity (Fig. 7c) could be observed due to the increase in ion concentration. Furthermore, the increase in ion concentration led to a considerable increase in the phase angle signal (Fig. 7b) and a modest but clear increase in the imaginary component of complex conductivity (Fig. 7d). This shows that in field soil not only root surfaces are polarized, but, in the case of high ion concentrations, polarization processes can also occur in the soil that are visible in the phase angle and the imaginary component even when the soil does not contain roots at this position. In figure 7d, in the lower right edge of the rhizotron, the imaginary conductivity was increased while at this position no roots were growing in the soil (Fig. 6a). 15 hours after infiltration of the fertilizer solution, the highest real conductivities (Fig. 7c) were observed in the lower part of the rhizotron. This movement probably occurred due to leaching and ion uptake by the roots in the upper part of the rhizotron. In the phase angle signal, this change was not visible (Fig. 7b) but it was visible in the imaginary component of the complex conductivity (Fig. 7d, decrease in the signal in the middle of the rhizotron). 10 days after the fertilizer application, when the roots had reached the bottom of the rhizotron, a strong increase in the magnitude resistivity (Fig. 7a) and a strong decrease in the real component of the complex conductivity (Fig. 7c) were visible. This change in the 2D distribution of the conductivity was most probably generated by ion uptake by the roots but especially by the daily irrigation with 100 ml of tap water in the last 10 days which led to a downward ion leaching. In the phase angle signal (Fig. 7b) an increase in the polarized area could be observed. However, in the
region where the conductivity was low (Fig. 7c) no increase in the phase angle signal (Fig. 7b) was observed while in the lower part of the rhizotron a polarization was visible in the phase angle signal, where at that time, ten days after fertilizer application, a modest real conductivity could still be observed (Fig. 7c). A similar modest increase could be observed in the imaginary component of the complex conductivity (Fig. 7d). The experiment shows that the tested amount of macropores does not affect the electrical impedance to an extent that they could mask the electrical impedance signal of the roots. However, changes in the ion concentration can lead not only to changes in the magnitude and the real component of the impedance, but also to changes in the phase angle and the imaginary component that are independent of root tissue.
Investigation whether roots can be detected in field soil. Three winter oilseed rape plants were dug out in the field (field trial CeFiT, DFG FOR 1320) in October 2011 and transplanted in a rhizotron filled with silty clay soil (topsoil) from the same field. The rhizotron was imaged using the EIT system Medusa II at 5 and 130 Hz. The roots of the three plants were completely surrounded by soil and did not touch the rhizotron or the electrodes.

a) Root length density (RLD) was determined using WinRHIZO. b) 2D magnitude tomograms at 5 and 130 Hz. The inhomogeneous distribution of soil moisture after a single irrigation with 500 mL of tap water was visible in the 2D magnitude tomogram, as the lower third of the rhizotron shows a high magnitude value (blue), indicating high resistance. Also, at the position where the roots were located, a high magnitude value was observed which originated probably from the thick taproots that had a low conductivity.

c) Polarization of the roots was visible in the 2D phase angle image. In the lower part of the rhizotron some structures in the phase signal can be observed at 130 Hz but much less at 5 Hz that are very likely related to the water content in the soil. This indicates that the phase angle signal is also affected by the water content of the soil, albeit to a much smaller extent than the magnitude signal. Data were presented in Kemna et al. 2011.
Figure 7. Experiment 3. Effect of air-filled macropores and infiltration of fertilizer solution on the impedance signal. Complex resistivity magnitude (a), phase (b), real component (c) and imaginary component of complex conductivity (d) of the oilseed rape plants in a rhizotron filled with silty clay soil that was already visible in Figure 7. In the text above the columns of images, the treatment of the soil is indicated. First, there were no pores pricked into the soil. Next, there were 15 pores (length: 14 cm, diameter 3 mm, volume per pore 1 mL) pricked into the soil neither touching the transparent plate nor the electrodes. The location of the pores is shown in figure (e). Afterward, there were 15 more pores pricked into the soil close to the first location (e), but this time directly along the transparent plate. Next, the pores were saturated with 300 ml of a 50% Hoagland solution. The next two columns show the results after 15 hours and 10 days later.
**Experiment 4: Wheat in field soil was irrigated with deionized water for 39 days and then fertilized**

In order to investigate in more detail how fertilization influences the complex resistivity response we irrigated a rhizotron filled with field soil containing six wheat plants (Fig. 8a) with deionized water (50 ml per day) for 39 days. After one week of watering (the total duration of the experiment was 39 days), we sowed the wheat seeds. The result after 32 days of plant growth for magnitude, phase angle, real component of the conductivity and the imaginary component of conductivity are shown in Figure 8 (b-e). All 2D images (magnitude, phase angle, real and imaginary component of complex resistivity) were dominated by the low electrical conductivity (Figs. 8b-e). Due to the irrigation with deionized water for 39 days the ion concentration was very low and the plants did not receive appropriate amounts of mineral nutrients any longer. The 2D distribution of the magnitude and the real component of the complex resistivity were lowest in the region with many roots (Figs. 8b, d) indicating uptake processes by the roots. The root distribution (RLD, Fig. 8a) had a visible effect on the phase angle and the imaginary component (Fig. 8c, e). However, this effect was indirectly visible. At the regions with many roots we did not observe the highest phase angle signal or imaginary component as we would expect when the root signal would be caused by the polarization of the root surface. Phase angle and imaginary conductivity were dominated by the real electrical conductivity, most likely driven by the ion uptake by the roots. Root systems were not polarizable themselves when the ion concentration in the soil was very low.

In a next step, we let 100 ml of a 0.625 mmolar ammonium nitrate solution infiltrate into the soil of the rhizotrons from above, and monitored the complex resistivity response for 48 hours (Figs. 8f, g). The fertilizer solution increased the real component of the conductivity in the upper part of the rhizotron (Fig. 8f). However, in the very upper part of the rhizotron no increase in the real component of conductivity was observed which was not comprehensible at first and could probably be explained by a very rapid ion uptake in the first two hours after the application of the fertilizer solution by the relatively high root length density in the upper part of the rhizotron (Fig. 8a). To our understanding, drying of the soil cannot occur that rapidly and intensely, and is therefore not the most likely explanation for this observation after two hours. In the region where a relatively strong increase in the real component of the complex conductivity occurred (Fig. 8f, strip-shaped red structure of high conductivity below the blue less conductive region), there
was also a strong increase in the imaginary component of the complex conductivity visible (Fig. 8g). This showed that a minimum ion concentration in the rhizosphere is necessary to make the root surfaces polarizable. A very systematic decrease could be observed within the next 48 hours in the real component of the complex conductivity (Fig. 8f) in the topmost part of the rhizotron. This effect cannot be explained by ion uptake alone: it is most likely due to drying of the soil caused by evapotranspiration, since no additional irrigation was performed after the application of the fertilizer solution. This decrease in the real component of the complex conductivity (Fig. 8f) also affected the imaginary component of the complex conductivity (Fig. 8g), albeit to a smaller extent. The results from experiment 4 with wheat in field soil (Fig. 8) confirmed the results from experiment 3 with oilseed rape in field soil (Fig. 7): both an increase in real conductivity due to an increase of the ion concentration and a decrease in conductivity due to a relative drying of the soil were visible in both the real and the imaginary component of the conductivity. However, comparatively small differences in ion concentration or the water content likely have only a relatively small impact on the imaginary component of the conductivity as could be shown in the experiment with oilseed rape in field soil (Fig. 7).
The first results, obtained with real field soil (experiments 2, 3 and 4) showed the importance of homogeneous soil conditions with respect to water content and ion concentration. We were interested whether it is possible to robustly monitor root growth over time when water content and ion concentration are kept as homogeneous as possible. Therefore, we have chosen a fertilized peat substrate (Graberde; Plantaflor Humus, Vechta Germany; containing N, \( \approx 120 \text{ mg L}^{-1} \); P\(_2\)O\(_5\), \( \approx 20 \text{ mg L}^{-1} \); K\(_2\)O, \( \approx 170 \text{ mg L}^{-1} \); pH...
5.4; electrical conductivity 1200 µS cm$^{-1}$) for this experiment, because peat is a common substrate in experiments with small containers in the laboratory since it allows for a homogeneous distribution of water and also strong root growth. Moreover, also real field soils commonly contain organic matter and substances similar to peat have to be expected in real field soils. For this reason, we wanted to test whether roots were also visible in such organic substrates. After the peat in the rhizotrons was adjusted to field capacity over several days, 16 oilseed rape plants were sown and EIT measurements as well as optical measurements of root growth were performed every other day for 29 days after sowing (DAS). The soil was watered automatically with 120 ml of tap water (500 µS cm$^{-1}$) per day in order to keep the soil as moist as possible. Results (Fig. 9) show that with the growth of the root system (normalized visible root length density (RLD), Fig. 9a), the phase angle signal (Fig. 9b) and the magnitude signal (Fig. 9c) clearly and systematically increased downwards. The average spatial similarities obtained for the comparisons between RLD (Fig. 9a) and phase angle (Fig. 9b) fell between 57% (minimal average spatial similarity) and 78% (maximum average spatial similarity). Furthermore, by the magnitude (Fig. 9c), the water content in the soil could be imaged as could be shown for the time of harvest (Figs. 9d, f). The average similarity of the 2D distribution of the magnitude signal (Fig. 9f) and the 2D distribution of the water content (Fig. 9d, in % VWC, determined gravimetrically by cutting the soil in 65 pieces of 6 * 6 * 2 cm in a 5 * 13 grid) was 95%. Using microsuction cups, the soil solution was sampled in the upper, middle and lower electrode rows of the rhizotrons, and the electrical conductivity was quantified for these samples (Fig. 9g). Changes in the electrical conductivity (EC) of the soil solution correlated with the 2D distribution of the magnitude (especially at the bottom of the rhizotron) but not with the 2D distribution of the phase angle. This indicates that the water content and the ion concentration dominate the magnitude but not necessarily the phase angle and polarization, respectively.

Experiment 5 shows that under controlled conditions, a systematic downward increase in the phase angle signal can be achieved that better correlates with root distribution than with water content or electrical conductivity of the soil. However, the phase angle signal was rather noisy and the 2D distributions of the phase angle signal changed with time which cannot be explained by root growth. Moreover, the experiment demonstrates that it is extremely difficult to keep the soil water content and the ion concentration constant over several weeks, even in rather small growth systems in the laboratory. In an additional experiment (data not shown), we irrigated with Hoagland solution of the same
electrical conductivity as the initial electrical conductivity of the soil solution, but we still observed gradients in the electrical conductivity of the soil. Processes such as ion leaching, filtering, ion uptake by roots and evapotranspiration inevitably lead to inhomogeneity in the named distributions of water content and ion concentration when roots grow over several weeks in soil-filled containers.
Figure 9. Experiment 5. Changes in complex resistivity magnitude (b) and phase (c) at 10 Hz of rhizotrons filled with “Graberde” (fertilized black peat substrate) in which 16 oilseed rape plants were sown on the left side. Rhizotrons were imaged every other day by EIT and the visible root length density (RLD) was determined every other day by GROWSCREEN-Root (a) for 29 days after sowing (DAS). At harvest (30 DAS) the soil was cut in 65 pieces (5×13 pieces) and the 2D distribution of water content (d) and the root length density of the roots washed out of the soil was determined (e). Note the similarity between the water content (d) and the magnitude (f). Note that the change in electrical conductivity (EC) of the soil solution measured in the upper, middle and lower rows of the rhizotrons (g) correlated with the 2D distribution of the magnitude (particularly at the bottom of the rhizotron), but not with the phase, indicating that the water content and ion concentration dominate the magnitude but not the phase. Data were presented in Pfeifer et al. 2012.
**Experiment 6: Decapitation of wheat in field soil and herbicide treatment of oilseed rape in peat**

Since residual roots from preceding crops can be expected in field soil, we studied the effects of dying and dead roots (devitalized roots) on the impedance signals in experiment 6. Figure 10 shows the result of a decapitation experiment (shoots were clipped off) and a herbicide experiment. Oilseed rape plants growing in fertilized peat substrate (Fig. 10a, b) were treated with a herbicide (33% Clinic-solution containing 120g L$^{-1}$ glyphosate; non-selective total herbicide) and were additionally decapitated. Wheat plants growing in field soil were only decapitated, but not treated with a herbicide (Fig. 10c, d). In both cases, independent of the soil (field soil or peat substrate) and independent from the killing method (herbicide treatment or only clipping off the shoot) the same result was observed: the signal of the real component (Figs. 10a, c) as well as the imaginary component (Figs. 10b, d) of the complex conductivity were paradoxically increased.

When the shoot is decapitated it can be assumed that some root cells may survive for some period of time (maybe even until the end of the experiment 33 days after decapitation). In contrast, roots that were treated with the herbicide likely contained much less living cells four weeks after the herbicide treatment. The death of the roots treated with the herbicide could be confirmed when the roots were washed out of the soil in the end of the experiment (the roots were strongly decomposed).
Figure 10. Experiment 6. EIT monitoring after devitalization of oilseed rape (a, b) and wheat plants (c, d) by herbicide application (a, b) and decapitation (shoots clipped off; c, d). Real (a) and imaginary component (b) of complex resistivity before and after the application of a herbicide (glyphosate) to oilseed rape plants growing in a fertilized peat substrate. The rhizotrons were watered to field capacity before each EIT measurement. Five days after application, the shoots of the plants were additionally clipped off. Real (c) and imaginary component (d) of complex resistivity before and after the decapitation of the shoots of wheat plants growing in unfertilized silty clay. The rhizotrons were continuously irrigated with 60 ml of water per day.
Experiment 7: Test of the influence of EIT measurements on plant growth

It is important that a non-invasive method for measuring plant growth not influence the growth itself or the physiological processes of the plant. Therefore, we monitored the generation of new leaves (leaf number), the generation of biomass of shoots and roots, and the spatial exploration of the soil by the roots (RSA pattern) in the course of experiments 5 and 6 (in experiment 6 some additional rhizotrons were not treated with a herbicide, EIT data not shown), for plants daily measured by EIT and plants that were grown under the same growth conditions but which were not measured by EIT. Within all our experiments no significant difference in root and shoot biomass production, development of new leaves and the RSA could be observed for plants measured daily by EIT and plants that were never measured by EIT (Table 1). As continuous EIT measurements did not lead to significant differences in growth and development of the treated versus non-treated plants, EIT can be considered a non-invasive method.
Table 1. **Experiment 7:** Comparison of root and shoot biomass production as well as developmental parameters from plants measured daily by EIT and plants treated identically but not measured daily by EIT from experiments 5 and 6 (in experiment 6 some rhizotrons were not treated with a herbicide, EIT data not shown). For average shoot biomass and average leaf number: arithmetic means ± standard error (SE). Using t-tests, no statistically significant differences were obtained for average shoot biomass (dry matter) per plant.

<table>
<thead>
<tr>
<th></th>
<th>Experiment 5 control rhizotron</th>
<th>Experiment 5 EIT-rhizotron</th>
<th>Experiment 6 control rhizotron</th>
<th>Experiment 6 EIT-rhizotron</th>
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<tr>
<td>Duration of the</td>
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<td>32</td>
<td>21</td>
<td>21</td>
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<tr>
<td>experiment (d)</td>
<td></td>
<td></td>
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<tr>
<td>Number of plants</td>
<td>16</td>
<td>15</td>
<td>18</td>
<td>15</td>
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<tr>
<td>per rhizotron</td>
<td></td>
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</tr>
<tr>
<td>Total shoot biomass</td>
<td>7.0</td>
<td>6.1</td>
<td>2.9</td>
<td>2.4</td>
</tr>
<tr>
<td>per rhizotron (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Average shoot biomass</td>
<td>0.44 ± 0.06</td>
<td>0.44 ± 0.09</td>
<td>0.16 ± 0.06</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>(dry matter) per plant (g)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total root biomass</td>
<td>1.0</td>
<td>0.9</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>per rhizotron (g)</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Canopy height</td>
<td>15</td>
<td>15</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>per rhizotron (cm)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Average leaf number</td>
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<tr>
<td>per plant</td>
<td></td>
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4.5 Discussion

A method that is capable of quantifying root growth in the field without destroying the soil-root system and therefore allowing continuous measurements is highly desired but lacking to date. In order to overcome this shortage we tested in the laboratory whether an EIT system with a high phase angle-accuracy could be applied for this task. Therefore, we tested on the one hand if it is possible to correlate the 2D distribution of root growth (root length density RLD: root length per volume element) with 2D EIT signals, and on the other hand we tested which other factors in the soil-root system might influence the electrical signals and possibly prevent the applicability of EIT to quantify root growth.
Polarization of roots in different ambient solutions

In the present investigation we tested in a first step the electrical spectra of roots in different solutions (without contact between roots and electrodes) and observed clearly distinguishable polarization responses of roots with Cole-Cole type signature (Cole 1932; Cole-Cole diagrams show the complex permittivity of dielectrics and real and imaginary component as a function of frequency) in the low-frequency range (phase angle peak between 1 and 10 Hz; Figs. 5a, d). This result clearly demonstrates that in principle, roots show a polarization response that is different from water and nutrient solution. This makes roots distinguishable from surrounding water. The correlation between root dry weight and the magnitude of overall polarization (normalized Cole-Cole chargeability) was almost linear and very strong (Fig. 5b). Using this calibration model it can be assumed that an increase in root biomass will lead to an almost linear increase in the overall polarization signal and that a quantification of root growth should, in principle, be possible via the polarization. However, when the roots are investigated in soil it is very important to consider other possible elements that might be charged within the soil-root system such as the surface of the soil particles. For example the polarization of soil particles may have a capacitance that is higher than the capacitance of the roots (Dietrich et al. 2013). For this reason we made the step from SIP in a small test cell to EIT in rhizotrons that were filled with soil from the field (Figs. 6, 7, 8, 10) and with fertilized peat substrate (Fig. 9) and investigated roots growing in soil.

EIT on roots in soil

Under conditions of a favorable supply of water and nutrients by the soil, and when the soil conditions were rather homogeneous, the spatial extension of root systems could be imaged both in field soil (Fig. 6) and in peat substrate (Fig. 9). However, when the conditions became less homogeneous (when the water content or the nutrient availability increased or decreased locally), serious difficulties occurred regarding the clear and robust differentiation of root polarization from other processes (Figs. 8, 10). These additional factors (water content, macropores, decapitated roots and ion concentration) that influenced the measurability of the root distribution in the soil were studied in more detail in the present work.
Effect of soil water content

The distribution of water content could be imaged very clearly by the magnitude and real component of the complex resistivity (Fig. 7d). This correlation was shown in several previous studies (Srayeddin and Doussan 2009, Amato et al. 2009). Very dry soil may mask the root polarization signal (data not shown). However, relatively small differences in the water content of the soil had a relatively small effect on the phase angle (Fig. 7a) compared with the magnitude (Fig. 7c) in the rhizotrons that contained oilseed rape plants sown in fertilized peat substrate. These results imply that the soil water content is, at least within a certain range, not a major problem for the interpretation of EIT results on root growth processes. Furthermore, since the water content was highly correlated to the magnitude, EIT is certainly useful for the imaging of the water distribution (and probably the water uptake) in the soil in crop research.

Effects of ion concentration in the soil

When the soil contained a low ion concentration (Figs. 8b, c, d, e) the polarization in the region with roots was not visible (Fig. 8c, e). When the ion concentration was increased in this system, the rhizosphere was polarizable again (Fig. 8g), but only in the region where the electrical conductivity was increased (Fig. 8f). Consequently, the root polarization signal might not clearly reflect the real extension of the root system (Figs. 8f, g) when the ion concentration is heterogeneous. Moreover, a strong increase in the ion concentration in the soil solution (as it was done for the experiment with the oilseed rape plants from the field, Fig. 7) resulted in a strong increase in the real component of the complex conductivity (Fig. 7c), and also in the imaginary component (Fig. 7d) also when there were no roots in this region of the soil. The phase angle signal was affected to a smaller extent (Fig. 7b). However, these results clearly show that the ion concentration is a very serious issue for the valid interpretation of EIT results. Maybe these results imply the opportunity that the nutritional status of the rhizosphere and nutrient uptake might be imaged and monitored using EIT. This might be an interesting application for crop breeding and management. However, for this application the water content of the field soil should be thoroughly controlled by precise irrigation.
Effects of artificial macropores

Macropores were not visible in the impedance signals (Figs. 7a, b, c, d). However, when fertilizer solution was infiltrated in the pores all impedance signals (magnitude, phase angle, real and imaginary component) were affected. This effect can be considered to be related to the ion concentration in the soil and is likely independent of the pores. Macropores are not a major factor disturbing the robust interpretability of EIT results from roots in the soil.

Effects of devitalization by decapitation and herbicide treatment

Decapitation of shoots (also when the roots were killed by a herbicide some days before and when the roots were certainly dead at the end of the experiment) led to a remarkable result: both signals, real component (Figs. 10a, c) and imaginary component (Figs. 10b, d) of the complex conductivity were strongly increased. The higher level of conductivity was maintained for at least four weeks. Probably, this effect can be explained by an increase in root surface due to decomposition or the release of ions and carbohydrates from the dying roots, and probably also by an increase in the population of the soil microflora that is fed by these substances. Probably, the cell walls of these microorganisms are polarized. In any case, the results show that not intact membranes of living root cells are responsible for the polarization in this experiment (6), but the existence of dead root tissue and the remaining rhizosphere can lead to a polarization as well. For the field scale, this result implies that heterogeneous soil conditions (for example those generated by roots of preceding crops) are a serious issue for the robust and valid interpretation of EIT results.

Implications for the interpretation of EIT data on the field scale

Our hypothesis that the root system itself and its surface in the soil is polarized to an extent that it is visible in the EIT images could not be corroborated by our data. Indeed, roots showed a very clear polarization in different ambient solutions (Fig. 5). However, root systems were not polarized when there was a very low ion concentration in the soil (Figs. 8a, b, c, d, e). When the ion concentration was increased in this system the
rhizosphere was polarizable again (Fig. 8g), but only at the position where the electrical conductivity was increased (Fig. 8f). This shows that the structure that is dominating the polarization signal consists of ions in the rhizosphere and not of the root tissue or the root surface itself.

Therefore, EIT might be applicable for an investigation of the ion exchange capacity of the root system, but rather not for investigating root system architecture itself. Yet, it has to be pointed out clearly that any quantification of ion concentration will be affected not only by ion exchange of the intact root with the soil, but also by root decomposition processes as shown by the herbicide experiment (Fig. 10): killing the roots led to a huge increase in the polarization signal which can be explained by the release of ions into the rhizosphere. However, the role of microorganisms in the polarization process is not known. The hypothesis from Dalton (1995) that the root surface that is in contact with the soil water is related to the capacitance signal could be confirmed by our data since very dry regions were not polarizable (data not shown). However, our data show that it is not only the soil water that is relevant for the signal, but that the ion concentration in the rhizosphere is of the same or even greater importance.

There are two problems that prevent the application of EIT for measuring root density in the field. First, in the field, it is still not possible to accurately predict the dynamic changes and spatial distribution of the water content and ion concentration over the course of the growth season, even when there are no roots growing in the soil. Many processes such as ion leaching, preferential water pathways and chemical and microbial processes make the soil doubtlessly to a very complex system (Kibblewhite et al. 2008). In case of roots growing in the field under study the system becomes even much more complex: it is impossible until today to predict the distribution of soil moisture (water uptake), distribution of ions and nutrients (nutrient uptake), and root turnover. Therefore, a correction (offset) of the polarization signal for these soil factors is not feasible to date because of the lack of basis on data.

Second, even when the distribution of the named factors (water content, ion concentration, dead roots) in the soil would be known (and even when their effect on the electrical impedance would be known), it would be very difficult to link a change in the polarization signal to one of the named factors and to filter out the proportion of the root signal on the overall signal. An increase in the polarization signal in a certain region could be generated for example by dead or dying roots, as well as hydraulic redistribution (passive transport of soil water by the root system from one location in the soil to another.
driven by water potential gradients in the root-soil interface (Prieto et al. 2012); e.g. hydraulic lift: redistribution of soil water from moist deep soil layers to dry upper soil layers by exudation of water by the root, Wan et al. 2000) or soil processes related to ion release (chemical or microbial). In contrast, a decrease of the polarization signal could be based on water or ion uptake by the roots or ion fixation (chemical or microbial). Therefore, it will be an enormous challenge in future studies to disentangle the multiple factors that leave their imprint on the EIT signal.

4.6 Conclusion

Our data imply that it will be an enormous challenge to apply EIT for the characterization of root system architecture or root growth. In field soils, not only the root mass or the activity of the intact root contribute to the determined polarization signal but also several soil factors (water content, ion concentration, dead roots) affect the polarization signal. This leads to a superposition and masking of the root signal by the named factors. Our results show that our stated aim (to non-destructively monitor root density in the field) will likely not be achieved by EIT. However, it is conceivable that a valuable characterization of overall properties of the soil-root-continuum can be achieved via EIT and that these can be linked to certain functions within this ecosystem, if a sufficient number of boundary conditions are known.

4.7 Acknowledgements

The presented work is part of the subproject ImpTom, funded by DFG within the research Unit FOR 1320 “Crop Sequence and Nutrient Acquisition from the Subsoil”. J.P. is grateful for stimulating discussions with Stephan Blossfeld, Fabio Fiorani, Philippe Hinsinger and Hinrich Lühring.
Chapter 5

Artificial pores attract barley roots and can reduce artifacts of pot experiments³

5.1 Abstract

Soil compaction is a severe agricultural problem. It is characterized by increased resistance to root penetration and by a decreased amount of porosity in the soil. Until today it is not clear whether crop roots are able to actively detect remaining pores in compacted soil, as there have been inconsistent results in previous studies. Moreover, little is known about the capability of roots to leave pores again, into which they have grown, if the mechanical resistance of the bulk soil allows so. The aim of this study was to investigate the root growth response of spring barley (Hordeum vulgare L. cv. Ascona) in different configurations of a compacted loamy soil containing pores. The three-dimensional configurations of the root systems from three well watered and fertilized treatments were analyzed by X-ray computed tomography. All soil-filled cylindrical plastic pots (diameter: 60 mm, height: 210 mm) contained loose topsoil but differed in subsoil structure. In treatment ‘Loose’ [L] the pots were entirely filled with loose soil. Treatment ‘Lower part compacted’ [C] contained compacted soil in the lower part of the pots. Likewise, treatment ‘Pores’ [P] contained compacted soil in the lower part too, but here 16 artificial pores (1 mm diameter) were generated in the central part of the compacted subsoil zone. Comparison of the two treatments with compacted soil [C] and [P] showed that the roots were able to actively detect pores. However, the roots frequently grew across the pores or left the pores again after having grown into them, leading to a significantly higher fraction of roots exploring the compacted soil in the treatment with pores compared to the treatment without pores. These findings are useful for designing controlled experiments in pots of limited size that can mimic root growth in relatively complex soil structures which are more similar to field situations than usual pot experiments.

³Submitted to: Journal of Plant nutrition and Soil Science
5.2 Introduction

Soil compaction is one of the most serious current problems in crop production and land use (Tracy et al., 2011). Especially ‘hardpans’ in the subsoil (i.e. soil beneath the ploughed soil layer) are a widespread nuisance (Raney et al., 1955; Horn et al., 1995; Hamza and Anderson, 2005). Soil compaction is an immediate problem for the farmer because it results in yield decline, but it can also lead to imminent risks and irreversible damages such as erosion events due to reduced infiltration. The generation of pores in compacted soil layers, for example by specific crops generating biopores with their roots in compacted subsoil or for example by deep mechanical loosening (The Profitable Soils Group, 2009), is assumed to aid crop roots to grow into deeper soil layers (Jakobsen and Dexter, 1988; Stirzaker et al., 1996; Gaiser et al., 2012; Kautz et al., 2013). Yet, it is difficult to design controlled experiments that investigate the benefits of such measures. Whether roots can profit from pores in compacted soil layers depends on chemical and physical soil properties, such as pore diameter (root-soil contact), concentration and distribution of nutrients in the bulk soil and the pore wall, content and distribution of water, filling of the pore (for example with dead root tissue), permeability for air, and air-filled porosity in the soil (Stirzaker et al., 1996; Stirzaker and Passioura, 1996; Passioura, 2002). Previous studies investigated whether roots seek pores (a behaviour called ‘trematropism’ = a tendency to turn into direction of a hole, here a pore) in impenetrably compacted soil with partly inconsistent results (Dexter, 1986; Stirzaker et al., 1996). Dexter (1986) investigated whether roots of pea and wheat plants enter artificial macropores (1.5 to 4 mm diameter) drilled in artificial subsoil made of perspex plastic. Dexter (1986) suggested that the roots might not be able to seek these artificial pores, but he was not able to prove his assumption ultimately. In the study of Dexter (1986) the shoot growth response was not investigated. In contrast, Stirzaker et al. (1996) used real field soil for their experiment and observed ‘trematropism’ for barley roots since the roots occupied large artificial macropores more frequently than expected by chance alone. Stirzaker et al. (1996) needed to open the pots and cut through the soil in a depth of 100 mm to be able to determine the exploration of the pores by the roots in this depth layer. Thereby, it is unavoidable to destruct the soil-root system and the associated soil structure. Moreover, only a small section of the entire soil volume could be studied. Due to these restrictions Stirzaker et al. (1996) were not able to follow single roots on
their path through the soil and study the sequences of growth decisions taken by the same root in situ in respect of pore structures. However, this is feasible when the root system architecture (RSA) is resolved in 3D and in situ. Recent advances in X-ray computed tomography make such investigations possible today (Tracy et al., 2012; Mairhofer et al., 2013).

Particularly in pot experiments using compacted natural field soil, the availability of oxygen is frequently poor due to small oxygen diffusion rates (Passioura, 2006). This problem may be increased by inappropriate irrigation protocols (Passioura, 2006). Moreover, in pot experiments roots growing preferentially along the pot wall can very frequently be observed (Gregory et al., 2003; Poorter et al., 2012). This behavior leads to border effects and can result in artifacts (Passioura, 2006; Poorter et al., 2012).

In this experiment, X-ray computed tomography was used to non-destructively study RSA traits of root systems and root growth decisions of single roots within defined soil samples in 3D. For this purpose, a system was developed that enabled a controlled investigation of the use of macropores by roots under largely realistic conditions (corresponding to field conditions). In future studies it will also be possible to investigate undisturbed soil samples taken from real field soils (Pagenkemper et al., 2013). In the present study the following research questions were tested: a) Do barley (Hordeum vulgare L. cv. Ascona) roots grow into artificially generated macropores (diameter 1 mm) which are pressed into compacted field soil, even when the compacted layer is penetrable by the roots and the supply of water and nutrients is sufficient? How do the roots grow inside the pores? Do the roots stay inside the pores, or do they leave the pores again? b) In case the roots seek the artificial pores in compacted field soil, do the pores reduce preferential growth along the pot wall and consequently diminish border effects? c) Does the presence of pores increase shoot growth?

5.3 Materials and methods

Treatments

The present investigation involved the comparison of three treatments (Fig. 1a): cylindrical pots entirely filled with loose soil: ‘Loose’ [L] treatment; pots containing compacted soil in the lower part and loose soil in the upper part of the pot: ‘Lower part
compacted’ [C] treatment; and pots filled according to treatment [C] but containing artificial macropores in the compacted soil: ‘Pores’ [P] treatment. For treatment [L] and [C] two pots and for treatment [P] three pots with four plants in each pot were used (for a total of n=8 and n=12 replicate plants, respectively).

In a first step, cylindrical plastic pots (Fig. 1b; Polyethylene 100, drinking water grade and softener-free; inner dimensions: 210 mm height, 60 mm diameter; external diameter 75 mm; closed by a welded bottom made of polyethylene) were filled with a 10 mm layer of perlite for better drainage and air exchange. The perlite layer was gently compacted by a pressure load of 0.06 MPa. Drainage was permitted by four holes of 1 mm diameter drilled all around into each pot in a height of 5 mm above the bottom. The perlite layer reached 4 mm higher than the draining-boreholes so that water could easily drain through the perlite.

![Fig. 1: Barley plants were exposed to different soil structure treatments within cylindrical plastic pots. (a) From left to right drawings illustrate the three treatments which were compared: ‘Loose’ [L], ‘Lower part compacted’ [C], ‘Pores’ [P]. Dots in the black circle on the right side illustrate the position of the 16 pores (white lines) within the compacted soil zone of treatment [P]. (b) Photo of a pot at 14 days after sowing.](image)

Next, pots were filled with field soil (Haplic Luvisol; silty loam; topsoil after two years of lucerne growth from research station of Klein-Altendorf (6°59′29″N, 50°37′21″E),
University of Bonn, Germany; soil was sieved to an aggregate size of 2 mm) with different compaction treatments. Treatment [L] was prepared by adding three portions of 200 g moist soil (16.8% volumetric water content) on top of the perlite layer, while each portion was compressed gently by a pressure load of 0.012 MPa before the next portion was added to the pot. By then, the pot was filled until 5 mm below the top. In treatments [C] and [P], the lower, compacted layer was realized by addition of 500 g of moist soil (16.8% volumetric water content) on top of the perlite layer. This soil portion was then compacted by applying a pressure load of 0.3 MPa. For treatment [P] 16 artificial pores were generated by means of pressing a sharpened wire (1 mm diameter) made from stainless steel into the compacted soil down into the perlite layer. (Thereby, it was excluded that capillary cracks were generated since the soil was moist, plastic and still compactable). The loose layer on top of the compacted layers from treatments [C] and [P] were adjusted stepwise by adding portions of 200 g of moist soil into the pot. The portions were compressed using a pressure load of 0.012 MPa until the pot was filled until 5 mm below the top.

Soil compaction and mechanical resistance

Average dry soil bulk density (loose soil: 0.90 g ml\(^{-1}\); compacted soil: 1.64 g ml\(^{-1}\)) was calculated from the weights determined while the pots were filled taking the gravimetrically determined soil water content into account. Soil water content was determined by drying three samples of moist soil (100 g each) as it was used for filling the pots for three days at 105°C in a drying oven. Mechanical resistance of the soil (loose soil in [L], [C] and [P]: 0.34 ± 0.1 MPa; compacted soil in [C]: 0.97 ± 0.3 MPa; and compacted soil in [P]: 1.41 ± 0.1 MPa, respectively; mean value and SE) was measured at the end of each experiment directly after destructive shoot harvest using a manual penetrometer (Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands) that had a 30° cone angle, an 8 mm maximal cone diameter and a penetration depth of 10 cm, and was fitted with 5-N and 50-N steel springs.

Plant cultivation conditions

Spring barley seeds (\textit{Hordeum vulgare} L. cv. Ascona) were germinated at 21°C in Petri dishes (Greiner Bio-One, Frickenhausen, Germany) on filter paper (MN 713, Macherey-
Nagel, Düren, Germany), which was moistened with deionized water (Milli-Q, Millipore Corporation). After two days of germination comparable seedlings with about five to seven seminal roots of approximately 8 mm length were selected. Four seedlings per pot were transplanted in a depth of 10 mm in shape of a square with an approximate distance from each seedling to the pot wall of about 10 mm. The soil was allowed to take up water for 24 h by filling a tray with 1.5 cm of a 0.2% Wuxal super-solution (N 0.016%, P$_2$O$_5$ 0.016%, K$_2$O 0.012%, trace elements; Manna, Herrenberg, Germany), in which the pots were placed. Here, the draining boreholes were just submerged so that the water could rise into the soil solely via the perlite layer. Afterwards, the pots were placed every second day for 24 h in the tray containing a refreshed Wuxal-solution. In this way, the soil was allowed to take up water and nutrients for periods of 24 h, and could drain and aerate for the following periods of 24 h, respectively. This 48 h cycle was repeated until the end of the experiment. Water was additionally supplied to the plants by spraying the soil surface with 10 ml of 0.2% Wuxal super-solution every day. The described watering procedure may have led at least temporarily to conditions of lower aeration in the compacted soil zones in treatments [C] and [P] due to a reduced number of draining pores when compared to loose soil.

Plants were grown in a controlled environment chamber at a photoperiod of 12 h / 12 h light / dark with a photosynthetically active radiation (PAR) of 300 µmol m$^{-2}$ s$^{-1}$ at initial plant height, a relative air humidity of 50/60% (day / night) and a temperature of 22 / 19°C (day / night).

Phenotyping of shoot growth and conventional determination of shoot development

Shoot growth was monitored over 14 days after sowing (DAS). Images of shoots were taken daily from 7 DAS using a conventional digital SLR camera (EOS digital 400D, 10 MP, Canon, Tokio, Japan; combined with a 28 mm EFS lens, Canon, Tokio, Japan) with a blue screen as background. Projected leaf area was calculated from images according to protocols described elsewhere (Nagel et al., 2012) using ImageJ software (ImageJ, NIH, USA). Shoot development was monitored by conventional observations counting tillers and leaves.
Analysis of the occupation of soil pores by roots and of the root system architecture by X-ray computed tomography

The root system architecture was imaged at harvesting time point (14 DAS) using X-ray computed tomography. Thereby, not the entire pot was imaged but the detail from the soil surface down to 163 mm below the soil surface. All pots were scanned at the Swiss Federal Institute of Technology Zurich (ETH Zurich, Switzerland) using a phoenix v\textversion{tomex}s 240 X-ray scanner (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany). CT images were acquired using the following acquisition parameters: scanning resolution: 50.0 µm voxel edge length; current: 450 µA; voltage: 180 kV; number of images: 3000; averaged images: 3 (skip: 1); filter: 0.1 mm copper; binning: none; exposure time per image: 200 milliseconds; detector exposure time: 40 minutes per subscan of 3 combined subscans per multiscan. Volume reconstruction was performed with the software datos\textversion{x} (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany). CT volumes were analyzed using Visual Studio Max 2.2 software (Volume Graphics GmbH, Heidelberg, Germany) while the original data were scaled down to unsigned 16-bit format for further data processing. As the ‘region-growing’ algorithm of the software was used to segment the root structures, root systems of individual plants could not be separated because the root systems touched each other. (To the knowledge of the authors no algorithm is available which could solve this problem). For this reason, the values of the root volumes (Fig. 2e) measured by Visual Studio Max 2.2 software relate to all roots in a pot and not to individual plants. It was intended to determine mean root densities in compacted soil zones. Due to this reason it was sufficient that the root volume (Fig. 2e) relates to all four plants per pot.

In order to determine the distribution of the roots in the lower part of the pots (10 to 16 cm below the soil surface), which were growing along the pot wall or in the center of the pots, respectively, the root volumes measureable with VG studio software in the 3D X-ray scans were compared. Roots in the outer 0 - 3 mm of soil volume were considered ‘roots growing near the pot wall’, and roots growing in the rest of the lower part of the pot were considered ‘roots growing not near the pot wall’, respectively.
Destructive analysis of plant growth and biomass

At 14 DAS shoots were harvested. Shoots were dried by means of freeze-drying and shoot dry weight was determined. At the same time point, roots were washed out and total root length (per pot) was determined using WinRHIZO (images with 400 dpi, Regent Instruments Inc., Canada). In addition, root dry weight was measured for each pot (separating individual plants was not feasible) after drying for three days at 70°C.

Net assimilation rates (NAR, increase in plant biomass related to leaf area and time) for the experimental period were calculated according to Poorter and Remkes (1990) from measurements of root and shoot dry weight and leaf area made 14 DAS. Since the root dry weight could not be determined for individual plants, the data for entire pots were used to assess the root dry weight per plant by dividing the root dry weight per pot through the four plants growing per pot. This value was then added to the shoot dry weight in order to estimate the total biomass per plant for calculation of the NAR. Root dry weight accounted only for about 21±1% of the total biomass per plant.

Statistical analysis

Linear mixed models with the factor ‘pot’ were performed by means of the SPSS statistical software package (version 17.0, SPSS Inc., USA). In all cases where normality and/or homogeneity in variance were not achieved, non-parametric Kruskal-Wallis tests or Friedman tests were used. Plotting of figures was performed using the statistical software package SigmaPlot 12.2 (SigmaPlot, Systat Software Inc., Richmond, CA, USA) and the R package (R 3.0.2, The R Development Core Team 2013, http://www.r-project.org/).

One individual plant from treatment [P] developed very poor (shoot dry matter of 0.030 g at harvest). This plant was identified as an outlier and excluded from statistical analysis.
5.4 Results

Shoot growth

14 days after sowing (DAS), the plants from the treatment ‘Loose’ [L] showed a significantly higher shoot biomass production (shoot dry weight) when compared with plants from the treatment ‘Lower part compacted’ [C] (Fig. 2a). Plants from the treatment ‘Pores’ [P] showed an intermediate shoot biomass production, which was neither significantly different to [L] nor to [C]. A similar result was obtained for the net assimilation rate (NAR, Fig. 2b), which was reduced for plants from treatment [C] when compared to plants from treatment [L], while plants from [P] were in between [C] and [L].

Plants from treatment [C] showed a significantly greater specific leaf area (SLA) compared with plants from [L] and [P] (Fig. 2c). This result was not due to changes in projected leaf area (Fig. 2d) but due to changes in shoot dry weight (Fig. 2a). 14 DAS, the developmental parameters of the shoot, the number of leaves and the number of tillers, were not significantly different between the plants from the different treatments.
Fig. 2: Effect of the treatments ‘Loose’ [L], ‘Lower part compacted’ [C] and ‘Pores’ [P] on shoot and root growth at 14 days after sowing (DAS). Shoot dry weight (a), net assimilation rate (b), specific leaf area (SLA; projected leaf area : shoot dry weight ratio (m$^2$ kg$^{-1}$)) (c), projected leaf area (d), root volume in the lower part of the pots (mean values and s.e., (e)) and percentages of root volumes in the lower part of the pots which were located near the pot wall and in the inner part of the soil, respectively (mean values, (f)). Boxplots ((a) – (d)) show median (black horizontal line), mean (dashed horizontal line), quartiles (grey box) and lowest value still within 1.5 times the interquartile range (whiskers). Different characters above boxplots indicate statistically significant differences ($P < 0.05$), linear mixed models; post hoc test: LSD-method; n=8 (treatments [L] and [C]) and n=11 (treatment [P]), respectively.
The root volume measured by X-ray computed tomography was not different in the upper part of the pots when the treatments were compared (data not shown). However, in the lower part of the pots in a depth between 10 and 16 cm below the soil surface, the root volume showed a trend to be different for the treatments (Fig. 2e). Plants from treatment [C] showed a smaller root volume in the lower part of the pots compared with plants from treatment [L] \( (P = 0.09) \). Moreover, a further observation indicated a benefit for root growth in the lower part of soil of the treatment [P] when compared to treatment [C]: plants from treatment [P] showed a lower percentage of ‘roots growing near the pot wall’ (59%) in the lower part of the pots compared with plants from treatment [C], where a higher percentage of roots (80%) was growing near or along the pot wall (Fig. 2f; \( P = 0.08 \)). Here, the soil volume near the pot wall accounts for 19% of the total soil volume of the lower half of the pots. Similar to the shoot growth traits (Fig. 2), the plants from treatment [P] responded more similar to plants from treatment [L] when compared to plants from treatment [C].

Figure 3 shows representative CT-images of the root systems from the three treatments: ‘Loose’ [L], ‘Lower part compacted’ [C] and ‘Pores’ [P], which were achieved via X-ray computed tomography. It was visible from the CT-images (and in animations from the 3D views of the roots, data not shown) that plants in treatment [P] had a higher root density in the lower part of the pot (compacted zone) in comparison with treatment [C] and that the roots from treatment [C] grew preferentially along the pot wall in comparison with treatment [P], where the roots grew also in the middle of the compacted zone. In these animations, the effect of the different preference of the roots to grow along the pot wall in treatments [C] and [P] was clearly visible. Roots from treatment [P] grew, more similar to treatment [L], less concentrated along the pot wall when compared to treatment [C].
Table 1: Contact parameters between pores and roots in treatment ‘Pores’ [P] determined by analysis of X-ray computed images from 14 DAS. Arithmetic means ± SE, for n=3 pots with 4 plants per pot and 16 pores per pot.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Percentage of roots per pot that grew into the compacted zone (%)</td>
<td>85.5 ± 3.0</td>
</tr>
<tr>
<td>b) Percentage of roots that grew into compacted zone and contacted the pores</td>
<td>49.0 ± 6.2</td>
</tr>
<tr>
<td>c) Percentage of roots that contacted a pore and then entered it</td>
<td>69.5 ± 1.6</td>
</tr>
<tr>
<td>d) Percentage of roots that left the pore again after they had entered it</td>
<td>60.0 ± 20.0</td>
</tr>
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Figure 3: Representative images of the root systems from the three treatments: ‘Loose’ [L], ‘Lower part compacted’ [C] and ‘Pores’ [P]. The arrows next to [C] and [P] indicate the level of the transition layer between the loose soil zone (above) and the compacted soil zone (below).
Despite the compacted zones in treatments [C] and [P] were penetrable by the roots (Fig. 3), the compacted zones obviously were a barrier for root growth since roots frequently grew horizontally on the transition layer after vertical growth in the loose soil layer above (visible in Fig. 3, treatments [C] and [P]). Nevertheless, root counts in the X-ray scans showed that 86 ± 3% (mean value and SE) of the roots that reached the transition layer finally grew into the compacted zone in treatment [P] (Table 1a). 49 ± 6% (mean value and SE) of the roots that reached the transition layer contacted the artificially generated macropores which were generated in the compacted zone in treatment [P] (Table 1b). 70 ± 2% of these 49% of roots entered the pore (Table 1c). However, this means that 30 ± 2.7% (mean value and SE) of the roots that contacted a pore did not enter the pore but grew over it (see Fig. 5b, c, d). 60 ± 20% (mean value and SE) of the roots left the pore again, after they had entered it (Table 1d). While leaving the pores, the roots showed spiral root growth forming narrow curves in the pores (Fig. 5b, c, d). Most roots (Figs. 4, 5) were likely not able to establish a high contact to the pore wall in the 1 mm pores since the root diameter was always smaller than 1 mm.

Following the root growth decisions taken by the same root in situ, trematropic root growth was observed in treatment [P] (Fig. 4; Fig. 5b, c, d). The roots grew curves towards the pores.
Figure 4: Example for a root growing under treatment ‘Pores’ [P] in the transition layer between the loose soil zone (rather coarse soil visible in images (a), (b) and (c) at the right side) and the compacted soil zone containing pores (more homogeneously grey soil). The series of images consists of cross-section views (slices) that show different depth levels starting from a higher level (closer to the top of the pot) in direction to a lower level (closer to the bottom of the pot). The number in the images indicates the depth level below the first image (0.00 mm). The root moved from pore to pore ((a) – (g)). The arrow in (h) indicates the path that the root has taken.
Figure 5: Behavior of roots in pores. (a) Root enters pore within compacted soil just below loose soil zone (coarse zone in upper part of images) and stays inside pore; views from side. (b), (c) and (d) show further examples for roots that contacted pores. The roots leave the pores after a few mm of growth inside the pores again; cross-section views. Series of images (slices) from upper left to lower right image showing different depth levels starting from a higher (closer to top of pot) in direction to a lower level (closer to bottom of pot). Numbers in slices indicate the depth of slice relative to first slice. Arrows show the paths of root growth.
5.5 Discussion

This study investigated the response of barley roots to artificial macropores that were pressed in compacted soil. The pot experiment was designed to address the question whether the roots actively seek the pores and whether root and shoot growth are affected by the pores.

In this experiment a pore diameter of 1 mm was chosen because this pore size corresponds to some pore classes created by preceding crops tested in previous studies (e.g. Gaiser et al., 2012) to increase the biopore density in compacted subsoils, such as tap roots of lucerne (Hakl et al., 2011), or oilseed rape (Goodman et al., 2001).

Effect of soil compaction on root and shoot growth

In our experiment, decreased root growth in the compacted soil (Fig. 2e; Fig. 3) resulted from increased mechanical resistance and probably additionally from reduced supply of oxygen to the roots, as gas diffusion rates frequently decrease if the soil is compacted due to reduced macropore volume. Indeed, roots of cereals can grow a few decimeters into anaerobic soil (Drew, 1983). However, roots growing in anaerobic soil are short, thick, twisted, and dark and have less root hairs compared to roots growing in well-aerated soil (Gilman et al., 1987). Such roots were not found in this experiment, which let severely anaerobic conditions appear unlikely.

In treatment [C], although only the lower part of the pot contained compacted soil, the soil compaction exerted a stress to the plant which was strong enough to reduce shoot biomass accumulation (Fig. 2a). Young et al. (1997) showed that leaf expansion of barley was decreased due to increased mechanical resistance of sand substrate even if the aeration was unaltered and the matric potential was not increased. This shows that mechanical resistance alone can affect shoot growth.

Trematropism - Did the barley roots seek the pores?

Results from previous studies concerning the question whether roots are capable to actively detect pores in the soil and seek them (a behavior called ‘trematropism’) have been inconsistent (Dexter, 1986; Stirzaker et al., 1996). Our results confirm the
observation of Stirzaker et al. (1996) for trematropism, as trematropic root growth was observed for roots from treatment [P] (Fig. 4; Fig. 5b, c, d; Table 1b). Stirzaker et al. (1996) explained the trematropic growth and the detection of the pores by the way roots grow: ‘the small fracture ahead of the growing root may lead to other zones of weakness in the soil’, such as pores. This explanation, that roots follow the ‘path of least resistance’, which might be commonly true under natural conditions, appears not likely for the pores in treatment [P], since the pores were pressed into the soil by a piece of wire. By this procedure, the soil around the wire is compressed and therefore more compacted than the bulk soil. This effect was visible in the CT scans (for example Fig. 4, Fig. 5; note decreased porosity around pores).

Moreover, also capillary cracks around the pores can be excluded in this experiment, because the soil was moist and plastic when the pores were pressed in the soil. In [P] the overall soil mechanical resistance was increased, when compared with the soil from treatment [C]. As the water content and availability in [P] should be comparable to [C], the only remaining explanation for the observed trematropism (and the lesser proportion of roots at the pot wall) is the different air composition or oxygen content in the soil close to the pores in [P], when compared to treatment [C]. The higher oxygen content close to the pores might have been sensed directly by oxytropism (Porterfield, 2002), or indirectly via changes in the composition of the microflora. With respect to our results it appears evident that barley roots have developed at least one additional mechanism to detect pores and do not always simply follow the ‘easiest course’ and ‘path of least resistance’. These hypotheses can be tested in further experiments, probably by ‘hiding’ the pores from the roots by diminishing the differences in the air composition. For this purpose, small quantities of nitrogen gas could be led into the pores from below (here through the perlite layer for example), in order to prevent oxytropic effects. The quantities of nitrogen gas led into the pores should be small so that still enough oxygen comes into the loose soil from the atmosphere by diffusion.

*Did the roots become trapped in the pores?*

In case of roots growing inside pores it has been observed that roots frequently cannot leave the pore to re-enter the bulk-soil again (the roots were ‘trapped’, Stirzaker et al., 1996). If the pore diameter was too large or the pore wall was too hard, roots buckle at the pore wall (Whiteley et al., 1982). This observation led to the interpretation that the root
may not be able to exert the force required to re-enter into the pore wall. The conditions and mechanisms involved in this question have been scarcely investigated. Stirzaker et al. (1996) reported on roots that were ‘trapped’ in large biopores (3.2 mm diameter) when the roots had a diameter thinner than 0.8 mm. Only very thick roots were able to penetrate the pore wall. In contrast, Kautz et al. (2013) observed that roots of barley growing in the field may be able to leave biopores (> 2 mm diameter) in moderately compacted field soil (silty loam, Haplic Luvisol). Kautz et al. (2013) investigated barley roots in compacted subsoil that were growing in both, the bulk soil and in biopores using the profile wall method. Since the root length density in the bulk soil increased in deep soil layers with less compacted soil, Kautz et al. (2013) assumed that the roots may have left the biopores. However, these authors could not directly observe roots leaving pores. For this reason, they concluded that more research is needed in this field using minimal-invasive approaches such as X-ray computed tomography. In the present study, using the same soil as Kautz et al. (2013) in the laboratory, we could indeed show that roots were able to leave pores with a diameter of 1 mm. These roots often ‘pushed themselves off’ the opposite pore wall (note the narrow growth angles in Fig. 5b, c, d), thereby obviously exerting the required force and adjusting a wide angle to penetrate the pore wall as perpendicular as possible. Spiral growth of roots (circumnutation) has been considered a behavior of roots to find the ‘easiest course through the soil’ (Simmons et al., 1995). Here, the roots showed this growth pattern to discover more beneficial growth conditions in the bulk soil.

As the roots frequently did not enter the pores (they only contacted them) or left the pores again, the role of root hairs to contact the pore wall can be considered less important in this experiment. However, under natural conditions root hairs in pores are likely much more important (for example if the pore wall of earthworm burrows contains high amounts of nutrients). Some chemical (e.g. ion concentration) and biological (microbial activity) properties of natural biopore walls created by roots or earthworms have been shown to be different compared to the bulk soil (Pierret et al., 1999; Pankhurst et al., 2002). Yet, as pores age, the pore wall properties might change (Kautz and Köpke, 2009) and differences may probably become diminished.
In treatment [P], a further indication for the roots to be attracted by the pores was observed. Compared with the other treatments [C] and [L], a lower percentage of roots growing near the pot wall in the lower part of the pots has been observed in treatment [P] (Fig. 2f).

Several previous studies describe roots growing in soil-filled pots which do not maintain growing in the bulk soil as soon as they contact the pot wall, but elongate along the pot wall (Gregory et al., 2003; Poorter et al., 2012). Poorter et al. (2012) argued that limitations in water and nutrient uptake as well as changes in soil temperature in pot experiments cannot fully explain reduced photosynthesis rates observed for plants growing in small pots. Using NMR imaging (MRI) Poorter et al. (2012) showed that the main fraction of the roots of barley grew close to the edge of 1.3 l pots. This may contribute to negative effects on plant growth and consequently result in artifacts, as at the edges of pots the environmental conditions are usually less favorable compared to the conditions in the middle of the pot. At the edges of a pot the roots may encounter the mechanical impedance of the pot wall and the temperature fluctuates stronger than in the middle of the pot (Passioura, 2006). Furthermore, water and nutrient uptake may differ due to higher root density. Artifacts can also occur with respect to root growth analysis, when the average root density in the soil is assessed. If a high fraction of the root system grows at the wall of the pot this can even lead to changes in leaf morphology (bonsai-effect) as the leaves remain small due to a lower number of cells (Passioura, 2002).

The phenomenon of preferential root growth at the pot wall can be generated by several effects. These effects can be acting at the same time. Preferential root growth at the pot wall can be observed not only in compacted soil, but also if the soil is rather loose (as observed in this experiment too: treatment [L]). If the soil is compacted in the pot, the aggregates can be shifted and fit into each other in the middle of the pot. This is not possible at the wall because the wall is incompressible. For this reason, possibly a higher fraction of small pores remain at the wall while the soil is compressed. However, in our CT-images, at a resolution of 50 µm, we could not observe a higher proportion of pores at the pot wall. Monshausen and Gilroy (2009) report in their review that roots encountering mechanical barriers grow along the barriers in order to get behind them. This means that roots do not generally show a negative thigmotropism (thigmotropism: growth into direction of a touched object). Similar observations of thigmotropic root growth have
been reported: Konôpka et al. (2009) studied maize roots penetrating compacted field soil clods and Rytter (2012) reviewed observations of roots growing concentrated around rock fragments in the field.

This study showed that it is possible to attract the roots of spring barley (*Hordeum vulgare* L. cv. Ascona) to the middle of the pot by additional, artificial pores. In experiments designed for the investigation of soil compaction under controlled conditions in pots, pores appear beneficial for creating more realistic, field-like conditions, since the roots can be attracted by the pores and the number of roots growing at the wall of the pot could be reduced. The higher presence of roots in the inner part of the pot of [P] compared to [C] means that a larger fraction of roots in [P] was situated in the bulk soil compared to [C]. Roots growing at the pot wall were attached to the wall material with half of their surface. Hence, the pores – even if they were left again – assured that the roots of treatment [P] were surrounded by the substrate to a high extent. In the field, this will also most likely be beneficial, since the compacted soils of intensively managed sites are often only moderately compacted, well watered and well fertilized. Moreover, roots in our experiments were provided with oxygen from the air of the artificial pore, which is one of the most critical physiological issues in compacted soil in the field (Schjønning et al., 2013). With respect to water and nutrient availability, the pore wall itself was not more beneficial in our study compared to the bulk soil. This is different in field situations, where the pore walls are often covered with organic material from earthworm feces or roots of previous crops that are mineralized by soil organisms. In future experiments, it will be interesting to realize pore structures with walls that resemble the field situation more closely.

### 5.6 Conclusion

Our results show that pores helped to attract more roots towards the center of the compacted soil. Thereby a larger fraction of the root was brought in contact with the bulk soil and diminished the border effects of the pot wall. The pores improved root and shoot growth even if the roots did not grow predominantly in the pores. Thus, the observations presented here might be a beneficial indication how to set up controlled experiments in the future that are more closely approximating field growth situations. The results might
even imply that also in the field, induced pores, either by mechanical loosening or by preceding crops, might ameliorate crop cultivation on compacted soils. In the present experiment only artificial pores of a single defined diameter were investigated. In future studies it would be interesting to investigate effects of pore diameter, of pore wall compaction and of pore wall biofilm composition. Moreover, it could be tested whether in later growth stages a different root growth behavior (root system development, root orders, RSA) can be observed by monitoring root growth over time. Additionally, the importance of root hairs in establishing contact to the pore walls should be considered.

5.7 Acknowledgements

This work was supported by Swiss National Science Foundation (SNF) within NRP 68 ‘Sustainable use of soil as a resource’. We want to thank all people that contributed to this work. In particular, Hendrik Poorter, Frank Liebisch, Luisa Last, Michael Mielewczik and Susanne Tittmann contributed with stimulating discussions. We are grateful to Patrick Flütsch for constructing and assembling the pots (cylindrical polyethylene containers).
Chapter 6
General discussion

In several previous studies it has been shown that specific RSA traits have a strong impact on yield and overall performance of crops such as maize, rice, bean and wheat, particularly under limiting (stress) conditions (for a review see De Dorlodot et al. 2007, Zhu et al. 2011 and Gregory et al. 2013). In addition to their application for crop breeding, plant phenotypic data are also valuable for basic research, such as modelling of rhizosphere processes. Rhizosphere models can be implemented in integrated field scale models. Such knowledge can contribute to improving the sustainability of cropping systems (Herder et al. 2010).

In plant phenotyping, soil-based, in-depth root phenotyping methods are particularly underrepresented. However, phenotypic data obtained by these methods are, for specific phenes, most likely more valid and more realistically transferable to the field than data acquired via in-vitro methods. Furthermore, soil-based methods make specific investigations on soil-root interactions possible, which cannot be achieved using in-vitro methods. For this reason, this thesis focused on soil-based methods and intended to contribute to the rapidly growing field of new methods in crop root phenotyping.

In this thesis, selected novel methods for crop root phenotyping in soil were established, applied and subsequently evaluated for their specific value in crop breeding and basic research. A series of relevant questions were investigated regarding the effects of soil properties on crop root growth processes. In particular, effects of heterogeneous soil compaction, heterogeneous nutrient availability, and spatial distribution of macropores on the soil exploration by roots were studied. Several previously established non-invasive root growth phenotyping methods were applied and in part further developed (rhizotrons (chapters 2 and 3), X-ray computed tomography (CT, chapter 5), and FDR-probes (supplement 1 in appendix)). Moreover, a new method, EIT, (chapter 4), still untested for root phenotyping, was evaluated for its applicability in characterizing and monitoring root growth (root density) in a minimally-invasive manner in soil and in the field.

Phenotyping is undertaken to provide data to improve and better understand practical cropping systems. Since laboratory methods simulate field conditions, results obtained by laboratory methods have to be considered as proxies for the real processes occurring in
the field. For this reason, field methods are also very important, because it is indispensable to prove whether the phenes detected in the laboratory are relevant in the field, both for breeding and modeling (Araus and Cairns 2013). Thus, it would be very useful if non-invasive and non-destructive phenotyping methods applicable in the field became available for direct monitoring of root growth processes in soil. Due to this need, one of the major aims of the present thesis was the development of electrical impedance tomography (EIT, chapter 4) for this purpose.

6.1 Field phenotyping

In chapter 4 it was shown that via EIT, which was considered promising for direct non-destructive root growth monitoring in the field (Araus and Cairns 2013), the task can most likely not be solved in the near future. Not only the root density, but also changes in soil water content, ion concentration and dead roots strongly affected the EIT signal. Due to the fact that these factors have to be expected in field soil, and that the intensity of these factors is subject to spatial and temporal variations, EIT does not appear appropriate for direct measurement of root growth processes in the field. This result, if confirmed in future studies, suggests that alternative methods have to be developed for this purpose. Researchers and breeders interested in direct measurement of root growth processes under various soil conditions must for the time manage the task with available destructive/invasive methods such as shovelomics (Trachsel et al. 2011) or pinboard methods Böhm (1979). Destructive field methods allow for measuring root system architecture (RSA) traits in the field at one point in time. For continuous estimations of rooting depth and root density, profile wall methods have occasionally been used in the field (Gaiser et al. 2012). Here, root growth data are collected from roots accessible at the walls of large holes, which are dug out in the field. However, it cannot be excluded that roots growing close to such bare walls show growth responses that are different to roots in undisturbed bulk soil, particularly due to plant damage, changes in soil temperature and due to soil hydraulic effects. Another method for continuous estimation of root growth processes (rooting depth, root density) are mini-rhizotrons. Mini-rhizotrons are cylindrical, transparent plastic tubes, inserted in the field soil, which roots grow along and can be imaged by mini-cameras (Smit and Zuin 1996, Faget et al. 2010). It has been shown that mini-rhizotrons have two major limitations: many RSA-traits cannot be
determined (Polomski and Kuhn 2002) and changes in root growth processes observed using mini-rhizotrons have been considered unrepresentative for natural root growth in undisturbed soil (Smit et al. 2000). In sum, the methodological situation for the field remains unsatisfactory.

Even though this thesis has shown, that EIT is likely not appropriate for direct measurement of root density in soil, EIT might be applicable for determining and monitoring root water uptake in a minimally-invasive manner in the field. In recent years it has been shown that electrical resistivity tomography (ERT) allows for measurement of water content in soil, as the water content was highly correlated with electrical conductivity (Michot et al. 2003, al Hagrey 2007, Amato et al. 2008, Srayeddin and Doussan 2009). Electrical conductivity is one component of the electrical impedance (besides the imaginary component) and therefore determined via EIT. In this respect, EIT, if properly calibrated, may be applicable for monitoring water uptake in soil (similar to FDR-probes, see supplement 1 in appendix) and probably nutrition status of the rhizosphere. For this reason, EIT could be useful for modeling specific root functions in the field (water uptake, nutrient uptake) with the advantage that, in contrast to inserting FDR-probes, no huge holes have to be dug in the field. Furthermore, in this thesis it has been shown that EIT most likely has no influence on plant growth, since no differences to non-scanned control plants were observed. Hence, EIT appears promising for application of minimally-invasive monitoring of root functions at field scale. However, the present thesis focused on monitoring root growth dynamics and root system architecture in soil as well as on investigating root-soil interactions. Due to this focus, further root phenotyping methods (rhizotrons, X-ray computed tomography) were established, applied and evaluated.

6.2 Laboratory based phenotyping

Until now, all methods for measuring root density in the field require a considerable input of manual labor. Laboratory methods for soil-based in-depth root phenotyping can, depending on the research question, offer effective alternatives. Strong points of laboratory methods for root phenotyping will be discussed in part 6.2.1 and weaknesses in part 6.2.2.
6.2.1 In-depth phenotyping for elucidation of soil-root interactions

From the scientific and methodological point of view, laboratory methods are usually more versatile than field methods, since the soil conditions are more controllable and/or easier to characterize in the laboratory than in the field. Compared to field methods, laboratory systems commonly have the additional advantage of being non-invasive and non-destructive. Methods such as GROWSCREEN-Rhizo (Nagel et al. 2012, chapter 2) or X-ray computed tomography (chapter 5) could be used in complement to field methods in order to deepen the basic understanding of root growth processes and to provide estimated values for the parameterization and calibration of field scale models (if properly calibrated and appropriate protocols are used). Furthermore, such methods could support mechanistic investigations regarding soil-root interactions valuable for crop breeding.

Results from chapters 2, 3 and 5 showed that phenotypic plasticity (with regard to root and shoot growth as well as RSA and root growth dynamics) of crops affected by heterogeneous soil structure can be characterized in the laboratory. As the phenotype results from the interaction between genes (gene expression, gene regulation and epigenetics) and the influence of environmental conditions, it can be assumed that high genetic variability in a population is likely to result in high phenotypic plasticity. Genetic variability is a pivotal prerequisite for breeding, due to the fact that genetic variability is the basis for selection. Phenotypic plasticity, reflecting the genetic configuration of the organism interfering with environmental influences, can therefore be used to improve the adaptation of cultivars to specific environmental conditions. If the specific environmental conditions of the field can be predicted, a cultivar could be chosen that is optimally adapted and adjusted for these conditions with regard to morphological and physiological traits (including root growth, RSA and root system development). Moreover, it would be possible to use breeding to optimize a cultivar’s ability to adapt plastically and dynamically with respect to morphological and physiological traits (including growth) to changing environmental conditions within the growing season. If the cultivar shows an advanced ability to respond to changing environmental conditions, this could increase water and nutrient uptake and therefore yield. Breeding for both optimally adapted cultivars and for cultivars that are able to acclimate dynamically requires the possibility of characterizing the performance (and growth limitations) of the plants under various conditions. This task is achieved via image based phenotyping methods.
In chapter 5 for instance, using X-ray computed tomography, barley roots were shown to be able to detect artificial pores, able to enter the pores or not, and, if they entered to pores, able to re-enter the bulk soil or not. A similarly versatile and plastic behavior of roots encountering structured soil has also been observed in previous studies using structured field soil in the laboratory. Konôpka et al. (2009) observed variations in root diameter, branching density, lateral root length and root morphological deformations of maize roots growing in field soil containing fine soil and clods. These morphological (RSA) changes were observed by Konôpka et al. (2009) not only when the roots encountered clods, but also after penetration of the clods, when the roots entered the fine soil again. Even more interesting is the question whether these responses are under genetic control.

In general, plasticity of root systems encountering structured soil could be interpreted as a form of morphological and phenotypical plasticity. Moreover, this plastic morphology could be interpreted strategically, since a diversity in the way roots explore structured soil could be beneficial for water and nutrient uptake if soil conditions change (for a review see De Kroon and Hutchings 1995). Diversified positions of roots exploring structured soil (for example if some roots enter pores and some do not) leads to different root-soil contact situations and exposures to easily penetrable or hardly penetrable soil, and to soil containing easily extractable water or soil with low matric potential. If the topsoil dries out in the course of the growing season, it may be beneficial for the plants if some roots grow in pores in order to guarantee rapid depth growth and reach available water in deeper soil layers (see Supplement 1 in Appendix, Gaiser et al. 2012). Besides having low water availability, dry soil is also frequently harder for roots to penetrate. For this reason as well, pores may be beneficial. However, if the topsoil stays moist and the subsoil is compacted and contains only small amounts of water available to the plant (the hydraulic conductivity is commonly low in compacted soil), it may be beneficial for water uptake if some roots stay in the loose topsoil or grow into the bulk soil. The soil-root contact area is higher in the bulk soil than in wide pores and consequently the water uptake from the bulk soil may be higher, even if hydraulic conductivity is low. Similar considerations are relevant for nutrient uptake. In this context it has to be considered that morphologic differences of RSA traits (such as root diameter) might change hydraulic properties of the roots themselves and therefore water transport properties.

Mechanisms involved in plastic and dynamic adjustments of RSA are poorly understood, but are most likely regulated by genetic control and involve hormonal signaling (for a
review see Cahill and McNickle 2011, Laskowski 2013). Alpert and Simms (2002) discuss environmental and organismal factors that determine whether adaptive and plastic responses are likely to be advantageous or disadvantageous for the plant. First, the extent to which the environmental conditions are predictable in the specific environment is critical. (Does the new condition remain for a long enough period that is worth adapting to it?) Second, in case the duration of the new environmental condition is more or less predictable, it is essential that the particular trait can respond rapidly enough to allow for benefit under the new condition (Alpert and Simms 2002). Third, some morphological traits are irreversible (this may be relevant for several RSA traits, such as root diameter, initiated lateral roots or root cortical aerenchyma). If the plant adapts irreversibly, this may lead to disadvantages in case of additional changes in environmental conditions. Trade-offs involved in plastic responses of RSA traits are to a large extent not understood (Hu et al. 2013). In this respect, soil-based in-depth phenotyping systems such as rhizotrons or X-ray computed tomography could be useful for addressing such mechanistic questions. A better understanding of underlying mechanisms and genetic and hormonal control promises an enormous potential for crop production when used to improve the performance of cultivars.

In chapter 2, the robot system named GROWSCREEN-Rhizo was presented that allows for an automated and simultaneous monitoring of root and shoot growth in soil-filled rhizotrons in the greenhouse. By means of GROWSCREEN-Rhizo, it is possible to achieve images from shoots and root systems of plants growing along transparent rhizotron plates. Thereby, in addition to dynamic and interim development of root and shoot growth being possible to record, with respect to the root system, RSA parameters can also be monitored (such as total visible root length, visible root length density over depth and root length per surface area of rhizotrons (root length density distribution in two dimensions), rooting depth, root system width, visible lateral root length, lateral root length density, convex hull area (smallest convex area that includes the root system visible at the transparent plate)). GROWSCREEN-Rhizo allows for taking images from all 72 rhizotrons in a fully-automated mode within a period of 1.2 hours. Therefore, the temporal resolution is sufficient to resolve responses of root and shoot growth to environmental influences that occur rapidly, or to track diurnal/diel or circadian variations and rhythms of root and shoot growth processes.

In chapter 3, split-root rhizotrons were applied to study effects of vertically heterogeneous soil compaction on root and shoot growth of barley in the climate
chamber. Soil compaction leads to several complex constraints to soil structure. In *in vitro*-systems, such processes and interactions with roots (hampered root and subsequently shoot growth, morphological changes) most probably cannot be mimicked realistically or validly. However, in the rhizotrons used in this thesis, morphological changes known from the field could be mimicked in the laboratory. For this purpose, peat was used as substrate to cultivate the plants because peat is considered a poor substrate with respect to nutrients, which allows for adjustment of a large range of nutrient availabilities. Peat reacts if the water content is not too low (causing hydrophobicity), in principle comparable to many mineral field soils. For instance, compacted peat shows increased mechanical resistance, lower hydraulic conductivity and lower matric potential. These changes are known to occur in mineral soils as well. For specific studies, if a low nutrient availability is relevant for the research question, peat can be an appropriate surrogate for field soil in rhizotron experiments. For other investigations the rhizotrons could be filled with field soil.

### 6.2.2 Drawbacks and limitations of laboratory root phenotyping methods

Upstream and downstream processes are decisive limitations on GROWSCREEN-Rhizo for application in phenotyping screening-sequences (also called ‘phenotyping chains’, Nagel *et al.* 2012) which aim for high throughput if applied for selection in breeding. Currently, it takes a considerable amount of time to fill the rhizotrons with soil, as the soil has to be accurately compacted to a defined compaction level in order to prevent soil settlement processes within the monitoring period. Moreover, the images of roots and shoots have to be processed by semi-automated image analysis software demanding manual corrections in order to achieve quantitative results of the present root systems (Nagel *et al.* 2012). Both process phases, before and after the actual measurement (soil-filling and image analysis), can probably be automated to a much greater extent in the future. These automations would increase the already high practicability and usefulness of this method.

Compared to X-ray computed tomography, rhizotrons offer a considerably higher throughput. However, the high throughput that is achievable using *in-vitro* systems cannot be reached via rhizotrons today. To give an example for the order of the
throughput: scanning one cylinder (60 mm diameter) via X-ray computed tomography by a resolution of 50 µm, as done in chapter 5, took about 2 hours (without subsequent analysis), while a rhizotron can be imaged via GROWSCREEN-Rhizo in about a minute. However, filling soil cylinders and rhizotrons to an adjusted compaction level still requires a lot of manual labor. Imaging a petri-dish filled with agar (Nagel et al. 2009), a growth pouch (Hund et al. 2009a) or root system in an hydroponic or aeroponic system (De Dorlodot et al. 2005) take, comparable to imaging time for a rhizotron, a minute or even less. Topp et al. (2013) for instance, phenotyped more than 1400 root systems of rice plants using a gellan gum medium (similar to agar but more transparent) filled in cylinders for detection of quantitative trait loci (QTL) controlling RSA. As the entire root system is visible in gel-based systems, hydro-/aeroponics and at the surface of growth pouches, the image processing and image analysis for RSA parameter extraction is much easier compared to that for rhizotrons. At the transparent rhizotron plates, parts of the root systems almost always grow in the soil and are therefore not visible at the transparent plate. Both factors have to be considered with respect to phenotyping: the time for taking the image and the time for analyzing the phenes of interest.

6.3 Conclusion

In this thesis rhizotrons and CT were used to test specific relevant research questions about the effect of soil structure (soil strength, soil compaction, macropores) on root and shoot growth of barley. Both methods offer specific advantages for in-depth soil-based phenotyping of root-soil interactions. EIT, a method still untested for root phenotyping, was not specifically applied for investigating soil-root interactions in this thesis because it had been shown that EIT is most probably not applicable for monitoring root density directly and independently from different soil conditions. Our results on EIT imply that for non-invasive crop root phenotyping at field scale, new ideas are required that may adapt technical principles from available laboratory techniques. However, since EIT is likely applicable to minimally-invasive monitoring of soil water content in the field, EIT appears promising for monitoring root functions in the field. All methods tested in this thesis, including EIT, can therefore contribute to calibration and parameterization of rhizosphere models.
Particularly in chapters 3 and 5, an impressive versatility of root growth and development of within one barley cultivar was observed. In case those responses are under genetic control, and genetic variability can be detected within different genotypes, the results indicate a phenotypic plasticity probably useful for barley breeding. Furthermore, several insights in root-soil interactions were achieved (e.g. compensatory adjustments to heterogeneous soil conditions and root growth responses to pores such as trematropism, mechanism of re-entering bulk soil out of pores). It could be shown that the laboratory methods used are applicable for future phenotyping, particularly for the study of root interactions with soil physical parameters.

The results achieved in this thesis will hopefully contribute to advancement in root phenotyping research and inspire new investigations and experimental setups. Yet, an enormous ‘phenotyping bottleneck’ still exists: there is an urgent need to develop, establish and apply phenotyping methods that effectively provide valid phenotypic data that can be correlated with genetic data for selection in crop breeding. Efforts to further develop root phenotyping methods, particularly with respect to automation, have to be continued. Nevertheless, several highly useful plant phenotyping methods are available today. The next milestone will be to expand the activities in crop root phenotyping in the near future in a way that will allow for beneficial use in crop breeding programs, enabling the generation of new crop varieties to meet the upcoming, multifactorial challenges of agronomy.
Appendix

Supplement I:

Evidence of improved water uptake from subsoil by spring wheat following lucerne in a temperate humid climate

Abstract

Dry spells during the summer period affecting water uptake and plant growth in central Europe may occur more frequently in the future due to climate change. Improving the ability of crops to take up water from deeper soil layers is a potential strategy to secure water supply. The objective of this paper is to report on the effect of different preceding fodder crops on root growth and water uptake of spring wheat from the subsoil. Water extraction and root length density during grain filling of spring wheat were observed between anthesis and maturity in six different soil depths (0–15, 15–45, 45–60, 60–75, 75–90 and 90–105 cm) and with four different preceding crops: 1 year of fescue (Fes1Y), 2 years of chicory (Chi2Y), 2 years of lucerne (Luc2Y) and 3 years of chicory (Chi3Y). While there was no difference in total water extraction by wheat in the four crop sequences, water extraction from the deepest layer (90–105 cm) was significantly higher after 2 years of lucerne (Luc2Y). This was consistent with the root length densities measured in the 90–105 layer, which were 82, 89 and 112% higher in Luc2Y as compared to Fes1Y, Chi2Y and Chi3Y, respectively. Results suggest that lucerne as preceding crop supports deeper rooting and higher rooting density of following spring wheat enhancing access to water in deeper soil layers in response to prolonged dry spells. Effects facilitating root penetration like improved soil structure and higher nitrogen availability after lucerne are discussed. We conclude that suitable crop rotations with lucerne might be a cost-effective adaptation measure to overcome drought stress.

Introduction

Climate change will most likely lead to an increase in the frequency of dry spells during the summer period in southern and central Europe (Calanca, 2007; IPCC, 2007; Jacobs et al., 2008). Water is the most limiting factor for crop productivity in Europe’s intensive cropping systems. Securing water supply will therefore be essential to maintain crop yields (Brisson et al., 2010). Water supply can be improved by irrigation, but this is an option only in few regions in Europe. Another strategy to maintain crop yields under water scarcity is to increase drought tolerance through breeding e.g. the development of crop cultivars with a deeper and more efficient rooting system (Challinor et al., 2010; Debaeke and Aboudrare, 2004). Kirkegaard et al. (2007) showed in a field experiment that the water use efficiency of subsoil water at post-anthesis is 2 times higher than the total post-anthesis water use. However, the expansion of the rooting system into deeper soil layers depends not only on the genotypic ability of the crop but also on the change of soil properties from the topsoil to the subsoil. Although soil properties and in particular subsoil properties are difficult to modify, some crops with deep taproot systems are potentially able to penetrate even compacted layers and to create biopores in the subsoil which may support root growth and water uptake from subsoil layers of the following crop. One of these crops is lucerne (Medicago sativa L.) (Abdul-Jabbar et al., 1982; Carof et al., 2007; Li and Huang, 2008).

Hence, the objective of this study was to investigate under a temperate humid climate the effects of two deep-rooting fodder crops (lucerne and chicory [Cichorium intybus L.]) in comparison with one shallow-rooting crop (tall fescue [Festuca arundinacea Schreb.]) on soil water content, root growth and water uptake from the subsoil of the following spring wheat in a field experiment.
Materials and methods

Design of field experiment

The investigations are based on a field experiment which was established at the Klein Altendorf experimental station 6°59’29”N, 50°37’21”E) of the University of Bonn in 2007. The climate is characterized by temperate humid conditions with maritime influence.

Table 1. Average physical and chemical properties of the soil in the four treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Depth (cm)</th>
<th>Silt %</th>
<th>Sand %</th>
<th>Clay %</th>
<th>Texture</th>
<th>pH</th>
<th>Bulk Density (g cm⁻³)</th>
<th>Initial soil moisture (m³ m⁻³)*</th>
<th>WP (m³ m⁻³)**</th>
<th>Corg %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fes 1Y</td>
<td>15</td>
<td>77</td>
<td>8.8</td>
<td>15</td>
<td>SiL</td>
<td>5.0</td>
<td>1.31</td>
<td>25.4</td>
<td>8.9</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>75</td>
<td>5.9</td>
<td>19</td>
<td>SiL</td>
<td>6.3</td>
<td>1.51</td>
<td>27.9</td>
<td>13.2</td>
<td>0.35</td>
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<td>63</td>
<td>7</td>
<td>30</td>
<td>SiCL</td>
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<td>1.56</td>
<td>30.2</td>
<td>14.0</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>68</td>
<td>4.1</td>
<td>28</td>
<td>SiCL</td>
<td>5.4</td>
<td>1.56</td>
<td>30.3</td>
<td>16.8</td>
<td>0.40</td>
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<tr>
<td></td>
<td>90</td>
<td>69</td>
<td>3.6</td>
<td>27</td>
<td>SiCL</td>
<td>7.4</td>
<td>1.56</td>
<td>35.0</td>
<td>18.7</td>
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<td>SiL</td>
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<td>30.9</td>
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<td>SiL</td>
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<td>4.2</td>
<td>33</td>
<td>SiCL</td>
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<td>1.55</td>
<td>32.2</td>
<td>17.5</td>
<td>0.49</td>
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<td>6</td>
<td>32</td>
<td>SiCL</td>
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<td>1.62</td>
<td>33.9</td>
<td>18.7</td>
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<td>1.60</td>
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<td>Chi 2Y</td>
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<td>7.7</td>
<td>18</td>
<td>SiL</td>
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<td>29.2</td>
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<td>1.62</td>
<td>33.0</td>
<td>19.8</td>
<td>0.45</td>
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<td>3</td>
<td>30</td>
<td>SiCL</td>
<td>7.4</td>
<td>1.61</td>
<td>33.6</td>
<td>18.9</td>
<td>0.36</td>
</tr>
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<td>Luc 2Y</td>
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<td>7.9</td>
<td>20</td>
<td>SiL</td>
<td>5.7</td>
<td>1.44</td>
<td>29.1</td>
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</tr>
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<td>5.9</td>
<td>28</td>
<td>SiCL</td>
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<td>1.56</td>
<td>29.5</td>
<td>16.1</td>
<td>0.50</td>
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<tr>
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<td>3.1</td>
<td>34</td>
<td>SiCL</td>
<td>6.4</td>
<td>1.52</td>
<td>32.6</td>
<td>18.7</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
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<td>4.8</td>
<td>34</td>
<td>SiCL</td>
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<td>1.55</td>
<td>29.6</td>
<td>19.0</td>
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</tr>
<tr>
<td></td>
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<td>4.1</td>
<td>30</td>
<td>SiCL</td>
<td>5.6</td>
<td>1.56</td>
<td>31.6</td>
<td>17.0</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*Soil moisture at twelve days after sowing (7th April)
** WP Permanent wilting point (1500 kPa)
Mean annual temperature is 9.6°C with average rainfall of 625 mm, relatively evenly distributed over the year. Winter rainfall usually exceeds largely evapotranspiration which leads to a regular replenishing of the water storage in the subsoil. Frequently, dry spells occur in summer during the growing period. In 2010, the decadal climatic water balance (calculated as the difference between rainfall and potential evapotranspiration) was zero or negative from April to mid of August except for the first decade in May (Fig. 1). A pronounced dry spell occurred from June 7th to July 25th, where accumulated precipitation amounted to 58 mm against 212 mm of potential evapotranspiration (Penman–Monteith) (Fig. 1). The occurrence of dry spells is mainly due to the proximity of the Eifel mountains which at times prevent clouds from reaching the plain of Klein–Altendorf. The soil at the field experiment has been classified as Haplic Luvisol (FAO, 1998), which is characterized by a silty clay loam texture with clay accumulation in the subsoil (between 45 cm and 95 cm soil depth) and a calcium carbonate rich horizon below 95 cm (Table 1). Estimated available water capacity approximated 207 mm down to a soil depth of 100 cm. As rainfall in the winter term 2009/10 (October to March) was 300 mm against a total potential evapotranspiration of 94 mm, rainfall had been sufficient to replenish the water storage in the subsoil (Table 1). Effects of four treatments, representing different cropping sequences with fodder crops on water uptake and root growth of spring wheat, were investigated in a randomized complete block design (Table 2). In order to simultaneously investigate the effects of fodder crops after one, two or 3 years of cultivation, the fodder crops were sown successively in the spring of 2007, 2008 and 2009, respectively. Seeding densities were 25 kg ha\(^{-1}\) (lucerne), 5 kg ha\(^{-1}\) (chicory) and 30 kg ha\(^{-1}\) (tall fescue). Treatments designated for 2 years and 1 year of fodder cropping were previously sown with spring rye (in 2007) and oats (in 2008). The size of each plot was 60 m\(^{2}\).
During the pre-cropping phase the fodder crops were regularly cut and chopped (three to four times a year) with a mulcher. The shoot biomass remained on the soil surface. Depending on the fodder crop, manual weeding was necessary several times in the first year to establish pure stands of the fodder crops. In order to avoid nitrogen limitations, which had been observed in the fescue stands sown in previous years, 50 kg ha\(^{-1}\) N (as calcium ammoniumnitrate, CAN) were applied to 1 year fescue (Fes1Y). Apart from that, no other fertilizer was added to the fodder crops. Before tilling, the shoot mass was mowed and the residues incorporated twice with a chisel plough. The soil was tilled with a mouldboard plough to a depth of 30 cm before spring wheat was sown with a density of 450 seeds m\(^{-2}\) on April 7th, 2010. In order to realize the same level of plant available nitrogen in all plots, CAN was applied with amounts of 15 kg ha\(^{-1}\) N (Luc 2Y) and 77 kg ha\(^{-1}\) N (Chi2Y, Chi3Y, Fes1Y) according to the amount of available soil nitrogen content in 0–90 cm soil depth in the four treatments. Fourteen days after sowing a combination of 100 g/l Fluroxypyr, 2.5 g/l Florasulam and 80 g/l Clopyralid was applied to control weeds. Anthesis of spring wheat was observed on June 20\(^{th}\). Both anthesis as well as most of the grain filling period were affected by the dry spell which occurred from June 7\(^{th}\) to
July 25\textsuperscript{th} (Fig. 1). Spring wheat was harvested on August 13\textsuperscript{th}, 2010 from subplots of 8 m\textsuperscript{2} and number of grains per ear was determined. Prior to harvest, the number of ears per square meter on the subplots had been counted.

<table>
<thead>
<tr>
<th>Year</th>
<th>Luc2Y</th>
<th>Chi2Y</th>
<th>Chi3Y</th>
<th>Fes1Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>rye</td>
<td>rye</td>
<td>chicory</td>
<td>rye</td>
</tr>
<tr>
<td>2008</td>
<td>lucerne</td>
<td>chicory</td>
<td>chicory</td>
<td>oats</td>
</tr>
<tr>
<td>2009</td>
<td>lucerne</td>
<td>chicory</td>
<td>chicory</td>
<td>fescue</td>
</tr>
<tr>
<td>2010</td>
<td>spring wheat</td>
<td>spring wheat</td>
<td>spring wheat</td>
<td>spring wheat</td>
</tr>
</tbody>
</table>

Table 2. Crop sequences under study.

Soil moisture monitoring

Twelve days after sowing, soil moisture was measured for each treatment by weighing five soil cores extracted at each depth. The same soil cores were used to determine the wilting point at 1500 kPa pressure head. Frequency Domain Sensors (10HS, Decagon devices INC, Pullman WA, USA) for soil moisture measurements were installed 17 days after emergence of the spring wheat. The soil moisture probes were installed at a depth of 15, 45, 60, 75, 90 and 105 cm depth with four replications per treatment. The output voltage of the sensors was continuously registered with data loggers (dataTaker DT50, DT500 and DT80, Thermo Fisher Scientific Australia Pty Ltd.) at a 30 min resolution. Regular measurements as considered in this study started in June just after the start of the dry spell. Sensor voltages were transformed into volumetric soil water content by using the following calibration equation, provided by the manufacturer of the probes:

\[
VWC = -1.92 + 0.00669 \, U + -0.00000737 \, U^2 + 0.00000000297 \, U^3 \quad (1)
\]

where VWC is the volumetric soil water content (m\textsuperscript{3} m\textsuperscript{-3}) and U is the sensor voltage (mV) (Campbell et al., 2009).
Calculation of water uptake during the dry spell

For comparing water uptake by spring wheat between layers and treatments during the dry spell, measured daily volumetric water contents in each depth, treatment and replication were transformed into average water content per six soil layers (0–30, 30–45, 45–60, 60–75, 75–90 and 90–105 cm soil depth) by calculating the average volumetric water content from the water content of the probe at the lower and the probe at the upper boundary of the layer, except for the first two layers (0–30 and 30–45 cm). For the 0–30 cm layer, it was assumed that the water content measured at 15 cm was a reliable estimate for the average water content in this layer. For the 30–45 cm layer, the water content at the bottom of the layer was measured and the water content at 30 cm soil depth estimated by linear interpolation between the water content at 15 and 45 cm soil depth. The daily total water content (in mm) was calculated by multiplying the thickness of each layer (in cm) with the average volumetric water content (m$^3$ m$^{-3}$). Then the maximum and minimum water content between June 27th and July 25th was determined for each treatment, layer and repetition.

Since, during the entire dry spell no percolation occurred (total rainfall was 39 mm, distributed over 7 events, but none of the small rainfall events reached 15 cm soil depth), and water evaporation from the dry topsoil layer was negligible, the difference between the minimum and maximum water content in each subsoil layer approximates the depth specific water uptake by the crop (Vandegriend and Owe, 1994).
Monitoring of root length density

Root-length density (RLD) of spring wheat was estimated at the end of July 2010 during grain filling with the profile wall method (Böhm, 1979; Köpke, 1979). The same treatments as used for the soil moisture monitoring were analysed with two field replications. An excavator was used to install a trench (depth: 1.8 m). A 100 cm wide soil profile wall was smoothened to maximum rooting depth and transversely to the plant rows with a spade and sharp blades. Roots exposed from the wall were removed by scissors. With a fine spray of water at 3 bar pressure and the use of a small toothed scraper a soil layer nearly 0.5 cm thick was washed away along the vertical wall of the soil pit. Thus, surface roots were exposed. A frame (inner dimensions 100 cm × 60 cm with grids (5 cm × 5 cm) was attached to the profile wall. Root length units (RLU) equivalent to 0.5 cm root length were recorded in each square. RLD (cm root × cm−3 soil) was calculated for each square according to the following equation:

\[
\text{RLD} = \frac{\text{RLU} \times 0.5 \text{ cm}}{5 \text{ cm} \times 5 \text{ cm} \times 0.5 \text{ cm}} \tag{2}
\]

Crop observations

Phenology of crops and leaf morphology (e.g. rolling of leaves) are an indicator for water supply and crop water status. Observations were carried out in all treatments twice during the growing period of spring wheat: (1) on June 18, 2010 at the beginning of anthesis and (2) on July 12, 2010 during grain filling. Percentage of pollinating plants, of plants with rolling top leaf and proportion of green surface on the top leaf were visually registered on two subplots per treatment.

Statistical analysis

Due to the fact that the amount of extracted water from each layer was not normally distributed, statistical analysis of the treatment means of extracted water was performed with the Man–Randall ranking test using SigmaPlot 11 (2010 Systat Software Inc.) software.
Results

Changes in soil water content between anthesis and maturity

Precipitation in the beginning of May 2010 during the vegetative phase was sufficient and well distributed. At the beginning of June, after a rainfall event of 23 mm on June 6, a dry spell of almost 7 weeks was observed (Fig. 1). During this dry spell, soil water in each treatment and depth decreased continuously due to the water uptake by the crop, except for soil depth 15 cm in the treatment Fes1Y, where infiltrated water from the rainfall event on July 3rd reached this depth. This rainfall event caused either a stop of the decrease of soil water content or a slight increase of soil water content (Fig. 2). In 15 cm and 60 cm soil depth, soil water content in all treatments reached its minimum at mid of July, indicating that the crop was not able to extract more water. On the other hand, there was still plant available water for the crop below 60 cm soil depth until mid of August in Chi2Y, whereas in the other treatments soil water content in 90 cm reached its minimum at the beginning of August or earlier (Fig. 2). In 105 cm soil depth, as in 90 cm soil depth, the absolute soil water content differed between the treatments, which was due to contrasting extraction by the pre-crops (Table 1). In 105 cm soil depth, the water content remained constant over the whole observation period, except for the treatment Luc2Y. In this treatment, wheat extracted water from the beginning of the soil water monitoring until the end of July when a rain event with 19 mm occurred. Thus, our data provide strong indications that spring wheat was only able to extract water from a soil depth of 105 cm between flowering and grain filling when growing after lucerne.
Water extraction from soil layers

The amount of water extracted by spring wheat during the observation period in the four treatments was highest in the top layer (0–15 cm) in all treatments. No significant differences between the treatments were observed (Fig. 3). In the deeper layers water extraction was lower with higher variability between the treatments and with lower variability within the treatments. However, again no significant difference was observed between the treatments except for the treatment Luc2Y in the 90–105 cm soil layer, where water extraction during the dry spell was almost as high as in the top layer. This had strong implications on the water extraction pattern of spring wheat among the different soil layers. In the Luc2Y treatment main water uptake during the dry spell took place in 0–30 cm and 90–105 cm soil layers, whereas in the other treatments most water was taken up from the four top layers (0–75 cm). A peculiarity was observed in the
Chi3Y treatment, where water extraction from the middle layers (45–75 cm) was higher than those from the topsoil during the dry spell.

Fig. 3. Water extraction from six soil depths under spring wheat after anthesis (June 27 to July 25, 2010) following four different pre-crop treatments (Fes1Y, 1 year fescue; Chi2Y, 2 years chicory; Chi3Y, 3 years chicory; Luc2Y, 2 years lucerne) (bars without letters and bars with the same letter within the same soil depth are not significantly different at $P < 0.05$).

Table 3. Phenological and morphological properties indicative for drought stress on spring wheat after four different pre-crop treatments during a dry spell of 30 days in 2010 between start of anthesis (June 18, 2010) and early maturity (July 12, 2010).

<table>
<thead>
<tr>
<th>Date (phenological stage)</th>
<th>Indicator</th>
<th>Lucerne 2Y</th>
<th>Chicory 2Y</th>
<th>Chicory 3Y</th>
<th>Fescue 1Y</th>
</tr>
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<tbody>
<tr>
<td>June 18 (anthesis)</td>
<td>Ears pollinated (%)</td>
<td>5</td>
<td>50</td>
<td>55</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Plants with rolling of flag leaf (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Green area in flag leaf (%)</td>
<td>90</td>
<td>40</td>
<td>30</td>
<td>35</td>
</tr>
<tr>
<td>July 12 (grain filling)</td>
<td>Plsants with rolling of flag leaf (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green area in flag leaf (%)</td>
<td></td>
<td></td>
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</tbody>
</table>
Maximum rooting depth

The observed differences in water extraction from the soil layers were not reflected by the root-length density distribution (RLD). RLD decreased with soil depth in all treatments except for Luc2Y, where RLD remained constant in the lower three layers (60–75 cm, 75–90 cm and 90–105 cm soil depth). RLD in the Luc2Y treatment was significantly higher in the two deepest layers (Fig. 4), and was nearly twice as high as in the other three pre-crop treatments (Fig. 5). In the upper soil layers, RLD was highest either in Fes1Y (0–15 cm), in Chi3Y (15–45 cm) or in Chi2Y (45–60 cm).

Drought stress and yield components

Rolling of top leaves of spring wheat was equally severe in all treatments at the end of the grain filling period (mid of July). However, the start of pollination as well as senescence of spring wheat was considerably retarded in the Luc2Y plots (Table 3). Mid of July, the flag leaves in the Luc2Y treatment were green on about 90% of their leaf surface, while in the other treatments yellow leaf area indicated degradation of chlorophyll in more than 50% of the flag leaf area. Yield of summer wheat after Luc2Y was highest, followed by Chi3Y, Fes1Y and Chi2Y (Table 4). Compared to Fes1Y and Chi2Y summer wheat yield was 13% and 27% higher after Lucerne, although the differences were not significantly different. The higher yield after Lucerne was due to highest number of grains per m² caused by a high number of grains per ear and a moderate number of ears per m².
**Fig. 4.** Distribution of root length densities (cm cm$^{-3}$) of spring wheat among the soil layers during grain filling in relation to three different pre-crops (Fes1Y, 1 year fescue; Chi2Y, 2 years chicory; Chi3Y, 3 years chicory; Luc2Y, 2 years lucerne) (bars without letters and bars with the same letter within the same soil depth are not significantly different at $P = 0.05$).
Table 4. Grain yield and yield components of spring wheat following four different pre-crop treatments (Fes1Y, 1 year fescue; Chi2Y, 2 years chicory; Chi3Y, 3 years chicory; Luc2Y, 2 years lucerne).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield (Mg ha(^{-1}))</th>
<th>Grain mass (g 1000(^{-1}))</th>
<th>Grains per Ear</th>
<th>Ears per m(^2)</th>
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<tr>
<td>Lu2Y</td>
<td>4.02</td>
<td>35.8</td>
<td>21.8</td>
<td>515</td>
<td>11227</td>
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<tr>
<td>Chi2Y</td>
<td>3.17</td>
<td>37.4</td>
<td>14.0</td>
<td>599</td>
<td>8379</td>
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<tr>
<td>Chi3Y</td>
<td>3.63</td>
<td>36.8</td>
<td>18.8</td>
<td>530</td>
<td>9938</td>
</tr>
<tr>
<td>Fes1Y</td>
<td>3.55</td>
<td>36.2</td>
<td>22.5</td>
<td>439</td>
<td>9866</td>
</tr>
</tbody>
</table>

Fig. 5. Relative root length density (cm cm\(^{-3}\)) of spring wheat in three different pre-crop treatments compared to the Luc2Y treatment (RLD = 100) within six soil layers (Fes1Y, 1 year fescue; Chi2Y, 2 years chicory; Chi3Y, 3 years chicory; Luc2Y, 2 years lucerne).
Discussion

Beneficial effects of lucerne on soil structure and nitrogen availability for following crops were frequently reported (Kautz et al., 2010; Riedell et al., 2009; Spera et al., 2009; Wang et al., 2008). In humid climates, biomass production and crop yield of subsequent crops were improved (Gregory et al., 2005; Kautz et al., 2010; Riedell et al., 2009). On the other hand, in semi-arid regions, lucerne as pre-crop bears the risk to reduce and limit available soil water down to 3 m depth for the following crops (Jia et al., 2009; Shen et al., 2009; Wang and Li, 2010). However, soil water extraction has never been monitored continuously over the rooting depth in following crops after Lucerne under temperate humid conditions in contrast to semi-arid regions. With the continuous monitoring of soil water content in our study, it becomes obvious that water extraction from deep soil layers (90–105 cm) by spring wheat after 2 years of lucerne was significantly higher compared with chicory or fescue as pre-crop. Two reasons may explain an improved water
extraction from the subsoil by the follow crops: (1) lucerne as pre-crop improves soil structure and creates macropores which facilitate root growth and the access to the water reserves in the subsoil and/or (2) higher nitrogen availability associated with increased biomass accumulation leads to enhanced supply of assimilates to the root system, which in the case of dry spells, extends faster to the moister subsoil. In both cases, extension of the root system to the subsoil is promoted, which is confirmed by the observations on root length density (Figs. 4 and 5). This is in line with results of Kautz et al. (2010) who found increased RLD of the following wheat crop in the subsoil after lucerne on a Haplic Luvisol close to the site reported here. However, the maximum observation depth in the experiments carried out by Kautz et al. (2010) was 92 cm and no data on water extraction had been collected. There are seasonal and inter-annual differences in rooting patterns, which could influence our observations on the effect of preceding crops on the subsoil rooting density of the following spring wheat (Luo et al., 1995; Xu et al., 2007). Besides the improved physical access and the higher availability of assimilates for root growth an additional stimulus like drought stress can urge the roots to accelerate root elongation and extend their root system, although the effect of this stimulus is cultivar specific (Guoth et al., 2010; Reynolds et al., 2007; Whitmore and Whalley, 2009). This stimulus may be amplified by the fact that, in the lucerne treatment, the upper soil layers seem to be more depleted compared to the other three treatments as shown in Fig. 6. There, it is obvious that in all treatments the largest amount of water was extracted from the top layers, except for the lucerne treatment, because the soil water depletion by spring wheat and the preceding lucerne had been higher than in the other treatments. In order to satisfy the crop water demand, spring wheat roots in the lucerne treatment had to extend to deeper soil layers, thus extracting equal amounts of water from the top and deep subsoil layers. Although Fig. 6 shows an equal total water extraction by spring wheat in the four treatments, it is likely, that, if deeper soil layers (>105 cm) had been observed, the total water extraction of wheat in the lucerne treatment could exceed that of wheat in the other treatments. The longer grain filling period as expressed by the higher proportion of green leaf surface in July (Table 3) gives some evidence to this assumption, because increased root mass of wheat cultivars in the subsoil is associated with increased transpiration and prolonged grain filling phase (Lopes and Reynolds, 2010). Sap flow measurements in adjacent Luc2Y plots in July confirmed that wheat maintained high transpiration levels throughout the dry spell and beyond (personal communication, Dr. Matthias Langensiepen). Grain mass of wheat was not influenced by the improved water supply
during grain filling. This is attributed to the fact, that wheat after Lucerne produced higher number of grains per meter square and the additional assimilates had to be distributed to a larger number of grains. Although Kirkegaard et al. (2007) reported higher water use efficiency of extracted subsoil water after anthesis, the much higher water use efficiency of total extracted water during the dry spell is another indicator that subsoil water extraction continued to be higher over the entire grain filling period until maturity (Table 4). Thus, wheat after lucerne was able to maintain grain mass in spite of higher number of grains per square meter. As a result, grain yield per hectare was highest after 2 years of lucerne, due to highest number of grains per square meter and similar grain mass compared to the other treatments. Further investigations which should include deeper soil layers and years with less pronounced dry spells are required to allow a generalisation of the obtained results.

Conclusion

Compared to chicory and fescue, lucerne as a preceding crop had a positive effect on water extraction by following spring wheat from the deep subsoil of a Haplic Luvisol. This observation was supported by higher root length densities in the deeper soil layers. The investment of assimilates into a deepening of the root system by the wheat crop was probably a reaction to the dry spell occurring during the grain filling period, which was facilitated by the combined effect of improved soil structure and higher nitrogen availability after lucerne. We conclude that suitable crop rotations with Lucerne could be a potential adaptation measure to improve drought tolerance of cereals. However, further research will be required to allow a generalisation of the obtained results to other soils and climatic conditions.

Acknowledgements

We are grateful for the provision of soil chemical and physical data by Stefan Pätzold and the technical assistance by Johannes Pfeifer, Reiner Lock, Christoph Oberdörster und Maximilian Weigand for installing and maintaining the soil moisture monitoring system. Funding by German Research Foundation within the Research Unit 1320 is gratefully acknowledged.
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List of abbreviations

2D: two dimensional
3D: three dimensional
*A. thaliana*: *Arabidopsis thaliana*
AOI: area of interest
CO₂: carbon dioxide
CT: X-ray computed tomography
d: day(s)
DAS: days after sowing
DAT: days after transplantation
DW: dry weight
EIT: electrical impedance tomography
ERT: electrical resistivity tomography
FDR: frequency domain reflectometry
Fig.: figure
FW: fresh weight
h: hour(s)
h² /h²: heredability
*H. vulgare*: *Hordeum vulgare*
K₂O: potassium oxide
LED: light emitting diode
min: minute(s)
M: molar
n /n: size of statistical sample (statistics)
N /N: statistical population (statistics)
O₂: oxygen
P: probability-value (statistics)
P₂O₅: phosphate
PAR photosynthetic active radiation
R² /R²: coefficient of determination (statistics)
RGR: relative growth rate (in % d⁻¹)
ROI: region of interest
RSA: root system architecture
SD / S.D./s.d./sd: standard deviation
SE / S.E. /s.e./se: standard error
SIP: spectral induced polarization

*T. aestivum*: *Triticum aestivum*
Tab.: table
t: time
μ: micro

*Z. mays*: *Zea mays*
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# Curriculum vitae

## Personal Data

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## Work experience

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<td>02/2010-02/2013</td>
<td><strong>Scientific assistant</strong> (PhD-student) Subproject ImpTom within research group DFG-FOR 1320 „Crop sequence and the nutrient acquisition from the subsoil”, Forschungszentrum Jülich GmbH, Institute for Bio- and Geosciences, Section Plant Sciences (IBG-2), Jülich Plant Phenotyping Centre, JPPC, Dr. Kerstin A. Nagel, Dr. Fabio Fiorani), Jülich, Germany</td>
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<tr>
<td>01/2010-02/2010</td>
<td><strong>Trainee</strong> Forschungszentrum Jülich GmbH, Institute for Bio- and Geosciences, Section Plant Sciences (Dr. Kerstin A. Nagel), Jülich, Germany</td>
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<tr>
<td>07/2009-12/2009</td>
<td><strong>Research assistant</strong> University of Bonn, Campus Klein-Altendorf (Renewable primary products, medicinal and spice plants, Prof. Dr. Ralf Pude), Germany</td>
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<tr>
<td>04/2008-10/2008</td>
<td><strong>Diploma student</strong> Practical part of Diploma-thesis, Bayer Crop Science, Institute for Herbicide Research, Herbicide Resistance Product Support (Dr. Hubert Menne) Industriepark Höchst, Frankfurt am Main, Germany</td>
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<tr>
<td>11/2006-11/2007</td>
<td><strong>Student research assistant</strong> of the Alexander von Humboldt-foundation fellow Prof. Dr. Rui Carlos Peruquetti, Department INRES/Animal Ecology, University of Bonn, Germany</td>
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<tr>
<td>04/2005-10/2005</td>
<td><strong>Trainee</strong> at the organic mixed farm „Schlebacher Hof“ in Rheinbach (dairy, arable crops) and at winery Resch, Wiltingen, Germany</td>
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<tr>
<td>2005-2007</td>
<td><strong>Assistant</strong> in winery Resch (harvest, winemaking), Wiltingen, Germany</td>
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## Education

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<th>Date</th>
<th>Degree/Role Description</th>
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<tr>
<td>01/2010-12/2013</td>
<td><strong>PhD-thesis</strong> Title „Elucidation of root-soil interactions of crops in space and time by establishment and application of novel image-based non-invasive root phenotyping methods”</td>
</tr>
<tr>
<td>2009</td>
<td><strong>Diploma-thesis</strong> for the degree of Diplom-Agraringenieur (Engineer in Agricultural Sciences) on June 16 2009; Title „Vergleichende Untersuchungen zum Resistenzmonitoring von Ungräsern im Reis am Beispiel von Echinochloa spp. und Leptochloa chinensis”</td>
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</table>
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