**Artificial macropores attract crop roots and enhance plant productivity on compacted soils
(STOTEN-D-16-02918)**

Tino Colombi^{1*}, Serge Braun¹, Thomas Keller^{2,3}, and Achim Walter¹

Abstract

The structure of compacted soils is characterized by decreased (macro-)porosity, which leads to increased mechanical impedance and decreased fluid transport rates, resulting in reduced root growth and crop productivity. Particularly in soils with high mechanical impedance, macropores can be used by roots as pathways of least resistance. This study investigated how different soil physical states relate to whole plant growth and whether roots grow towards spots with favourable soil physical conditions. Experiments were conducted under controlled and field conditions. Soybean (*Glycine max* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) were grown on uncompacted soil, compacted soil and compacted soil with artificial macropores. The interactions between roots and artificial macropores were quantified using X-ray computed tomography. Active growth of roots towards artificial macropores was observed for all three species. Roots grew either into macropores (predominantly in maize) or crossed them (predominantly in wheat). The presence of artificial macropores in compacted soil enabled all three species to compensate for decreased early vigour at later developmental stages. These results show that roots sense their physical environment, enabling them to grow towards spots with favourable soil conditions. The different kinds of root-macropore interaction indicated that macropores serve as a path of least resistance and a source of oxygen, both resulting in increased crop productivity on compacted soils.

Keywords: *Root-soil interactions, Soil compaction, Soil functions, Soil physical fertility, X-ray computed tomography*

1. Introduction

An estimated 33 million hectares of arable land in Europe and 68 million hectares worldwide are degraded due to soil compaction (Batey, 2009; Hamza and Anderson, 2005; Van den Akker and Canarache, 2001). This soil compaction, which is mainly caused by heavy agricultural machinery (Tracy et al., 2011), results in increased soil bulk density and decreased pore connectivity. In particular, macropore volume is reduced in compacted soils (Bottinelli et al., 2014; Chen et al., 2014). Bulk density and porosity are relatively simple measurements to quantify soil compaction, but do not describe actual soil functions. It has been proposed that soil physical functions such as mechanical impedance, gas diffusivity, air permeability and hydraulic conductivity are more suitable to relate soil compaction and plant productivity (Colombi and Walter, 2016; Stirzaker et al., 1996).

Due to their decreased void space, which is needed for displacement of soil particles, compacted soils exert higher mechanical impedance than uncompacted soils (Hernandez-Ramirez et al., 2014; Suzuki et al., 2013; Valentine et al., 2012). Furthermore, decreased macroporosity and pore connectivity cause decreased water infiltration rates and hydraulic conductivity (Chen et al., 2014; Kuncoro et al., 2014a; Lipiec and Hatano, 2003) as well as lower gas diffusivity and air permeability (Kuncoro et al., 2014a; Scholl et al., 2014; Stirzaker et al., 1996). This increases the risk of anaerobic conditions in compacted soils compared with uncompacted soils (Tracy et al., 2011). Due to less plant available water in compacted soils (Lipiec and Hatano, 2003), evapotranspiration dries out compacted soils more likely than uncompacted soils, which further increases penetration resistance. Increased mechanical impedance and decreased fluid transport rates decrease root growth and can both be summarised as loss of soil physical fertility (Abbott and Murphy, 2007) caused by compaction. Decreased root growth leads to restricted access to belowground resources and therefore decreased plant productivity (Batey, 2009; Downie et al., 2012; Hamza and Anderson, 2005; McKenzie et al., 2009; Tracy et al., 2011; Walter et al., 2009).

Besides decreased root growth, soil compaction causes tremendous alterations to the root phenotype. Decreasing numbers of axial and lateral roots in response to soil compaction have been observed for

several crops, including small-grain cereals, maize and soybean (Coelho Filho et al., 2013; Colombi and Walter, 2016; Grzesiak et al., 2014, 2013; Pfeifer et al., 2014a). Furthermore, roots from compacted soils have been reported to show greater root diameter, larger root cortical area and increased abundance of root cortex aerenchyma compared with roots grown in uncompacted soil (Alameda and Villar, 2012; Colombi and Walter, 2016; Iijima and Kato, 2007; Lipiec et al., 2012; Siczek et al., 2013). In most of those studies, such changes in root phenotype were related to decreasing plant vigour (Colombi and Walter, 2016; Grzesiak et al., 2014, 2013; Pfeifer et al., 2014a; Siczek et al., 2013). Furthermore, it has been shown that legumes are more sensitive to soil compaction than grasses (Arvidsson and Håkansson, 2014) and that the tolerance to mechanical impedance differs between species (Bushamuka and Zobel, 1998; Busscher et al., 2000; Grzesiak et al., 2014).

Despite this knowledge on the responses of plants to compacted soils, relatively little is known about how roots interact with spots within the soil that provide more favourable or more hostile conditions for growth. Detailed information about the relationships between soil physical properties and whole plant development is needed to identify ecophysiological processes limiting plant growth under compaction. Such information can help in developing mitigation strategies for compacted soils (Hamza and Anderson, 2005), since plant roots are among the major drivers for the recovery of soil functions after compaction. Roots contribute to this structural recovery by creating new macropores due to bioturbation and acceleration of localized soil drying which can result in crack formation (Bottinelli et al., 2014; Dexter, 1991; Scholl et al., 2014).

It has been proposed that roots sense their surrounding environment not only by direct contact with the soil matrix or soil solution (Passioura, 2002). The term ‘trematotropism’ was introduced by Dexter (1986) to describe the ability of roots to sense holes from a certain distance and grow towards them to exploit them as paths of least resistance. Porterfield and Musgrave (1998) used the term ‘oxytropism’ to describe growth of pea roots towards higher concentrations of oxygen. Trematotropism and oxytropism can both be interpreted as an ability of roots to adjust to soil heterogeneity and avoid unfavourable soil conditions. For example, it has been shown that roots of different wild grass species

avoid obstructions in the form of large pieces of gravel (Semchenko et al., 2008). A number of studies investigating the interaction between roots and macropores under laboratory and field conditions have concluded that roots use either naturally occurring macropores (Athmann et al., 2013; de Freitas et al., 1999; Han et al., 2015; Kautz et al., 2013; Stirzaker et al., 1996; White and Kirkegaard, 2010) or artificial macropores that were experimentally inserted into the soil with steel rods (Nakamoto, 1997; Stirzaker et al., 1996) as pathways to reach deeper soil layers. This behaviour of roots in following the path of least resistance is more pronounced if the impedance of the surrounding bulk soil is increased (de Freitas et al., 1999; Kautz et al., 2013; White and Kirkegaard, 2010). Some studies have concluded that such root-pore interaction must result from active growth of the roots towards the pores, rather than from random growth (Stirzaker et al., 1996; White and Kirkegaard, 2010).

However, the methodologies used in these studies have their limitations. For example, endoscopy only allows rather large pores (>5 mm) to be investigated, while measurements along a profile wall or of slices of soil only permit quantification in two dimensions. Non-destructive imaging approaches such as X-ray computed tomography (CT) or magnetic resonance imaging are promising tools to overcome these limitations. Using these techniques, root traits have been quantified at sub-millimetre resolution in pots (Metzner et al., 2015) and in undisturbed samples excavated from the field (Pfeifer et al., 2015). In such a pot study it was observed that barley roots predominantly cross artificial macropores, rather than growing into them (Pfeifer et al., 2014b).

Another methodological limitation is that studies investigating the response of plants to compaction and macroporosity are often performed with young plants (Nakamoto, 1997; Pfeifer et al., 2014b; Stirzaker et al., 1996). This allows only immediate effects of soil physical functions on plant performance to be observed and not effects arising later during plant development. Combined assessment of soil physical functions, root-soil structure relationships and sophisticated shoot phenotyping is necessary to identify soil physical phenomena controlling plant development and drivers for soil recovery after compaction.

The aim of the present study was to determine how soil compaction and the presence of vertical macropores in compacted soil affect whole plant development. This was done by: i) quantifying the

effects of soil compaction and the presence of artificial macropores in compacted soil on crop development and performance and ii) investigating whether and how crop roots interact with artificial macropores in compacted soil. Experiments were conducted with soybean, winter wheat and maize in a growth chamber (young plants) and in the field (mature plants), representing a controlled and natural field environment, respectively. Plant-pore interactions were quantified using X-ray micro CT and a set of soil physical properties and plant vigour traits were determined.

2. Material and Methods

2.1. Plant material and root system properties

The crop species used in the study were: soybean (*Glycine max* L., variety Merlin), winter wheat (*Triticum aestivum* L., variety Arina) and maize (*Zea mays* L., variety LG 30.222). Beside their importance for global agriculture, these crops were chosen due to their differing root systems. Soybean is characterised by a taproot system, whereas wheat and maize have a fibrous root system with roots of rather small and large diameter, respectively.

2.2. Experimental set-up and growth conditions

Experiments were conducted under controlled conditions in a growth chamber and in the field. The soil (Pseudogleyed Cambisol) used in the growth chamber experiments was taken from the same site at Agroscope Zurich, Switzerland (8°31'E, 47°27'N, 443 m above sea level) where the field experiments were conducted. Soil texture consisted of 25% clay, 50% silt and 25% sand with 1.7% organic carbon and pH (CaCl₂) of 6.9 in the top 20 cm.

2.2.1. Soil columns for growth chamber experiments

Soil was excavated from the top 15 cm of the soil profile, dried to approximately 22% gravimetric water content, homogenised and sieved through a 2 mm sieve. Plants were grown in PVC columns of 4.9 cm inner diameter and 15 cm height. The bottom of the columns was closed off with filter paper and a 1 cm quartz-sand layer (particle size 0.2-1.7 mm) was added. On top of the sand layer, soil was packed in six layers of 2 cm height to a bulk density of 1.3 and 1.6 g cm⁻³, representing loose (Ctrl) and compacted (Com) soil, respectively. The surface of each packed layer was slightly-abraded to ensure homogeneous packing. In a third soil treatment, densely packed soil (1.6 g cm⁻³) was vertically

perforated five times with a stainless steel wire of 1.25 mm diameter to create artificial macropores (ComAP). The artificial macropores were allocated in a cross-like pattern (Figure 1a).

Seeds were germinated at 25°C for 48 h and selected according to homogeneous length of radicle and seed size. In every column, 1 cm of loose soil (1 g cm⁻³) was added on top of the packed layers and a 1 mm wide and 5 mm deep hole was made in the centre. The seed with emerged radicle was placed into this hole and covered with 0.5 cm loose soil (0.8 g cm⁻³). Before planting, soybean seeds were coated with rhizobia inoculum (*Bradyrhizobium japonicum*; HiStick; Becker Underwood, BASF, Little Hampton, United Kingdom). Each soil treatment (Ctrl, Com and ComAP) was replicated four times for each crop species.

All plants were grown for 20 days in a growth chamber with a day/night cycle of 14/10 h at 59% relative air humidity and an average temperature of 20.6°C. Average incident light was 417 (±20, s.e.m.) μmol s⁻¹ m⁻². Columns were weighed and watered daily to keep the soil water content constant at a level corresponding to a matric potential of -100 hPa, which represents a typical value for field capacity (Schjonning and Rasmussen, 2000).

2.2.2. Field experiments

Field experiments were conducted on the Soil Structure Observatory (SSO) at Agroscope in Zurich, Switzerland. The SSO was established in spring 2014 as a long-term field experiment with compacted (Com) and uncompacted (Ctrl) plots arranged in three blocks to study recovery of compacted soil. In March 2014, plots were compacted using a heavy agricultural vehicle with four wheels and 80 kN wheel load (for detailed information see Colombi & Walter (2016)). Tillage treatments varied slightly between crops (Supplementary data Table S1).

Soybean seeds were inoculated with *B. japonicum* before sowing on 20 May 2014. Until harvest on 24 October, mean day and night temperature was 21.3°C and 11.9°C, respectively, and total precipitation was 528 mm. Winter wheat was sown on 11 November 2014 and harvested on 4 August 2015. Until harvest, average day and night temperature was 13.7°C and 5.2°C, respectively, and 736 mm of

precipitation were recorded. Maize was sown on 27 May 2015 and experienced mean day and night temperature of 24.2°C and 13.3°C, respectively, until harvest on 28 September. Cumulative precipitation from sowing to harvest was 262 mm. Sowing density and fertilisation rate were similar to on-farm conditions (Supplementary data Table S1).

In the compacted plots of all crops, artificial macropores (ComAP) were created shortly after germination in five or six 0.25 m² squares along a 2 cm by 2 cm grid (Figure 1b), using the same stainless steel wire as in the growth chamber experiments. In the context of soil management, soil perforation could be seen as an alternative approach to commonly applied tillage operations in order to create potential pathways for roots and to improve soil drainage and aeration. Perforation depth was around 30 cm for soybean and wheat and, due to relatively dry soil, 20 cm for maize. Roots and shoots were sampled 91 days after sowing (DAS) for soybean, 218 DAS for wheat and 70 DAS for maize. This sampling time corresponded to the stage of major grain filling in soybean and to flowering stage in wheat and maize.

2.3. Soil physical properties

Soil physical conditions in the growth chamber experiment were determined from packed soil samples of 100 ml volume and 5 cm height. Four soil cores per treatment (Ctrl, Com and ComAP) were packed in 1 cm layers with sieved soil to a bulk density of 1.3 and 1.6 g cm⁻³. Five artificial macropores were made in the ComAP samples in the same pattern as described above. The samples were slowly saturated from below for two weeks and then equilibrated on a ceramic plate to a matric potential of -100 hPa. Air permeability (K_a [μm²]) and relative gas diffusion coefficient (D_p/D_0 [-]) were measured and calculated according to Martínez et al. (2016). Mechanical impedance was measured by two penetrometer insertions into the bottom end of the soil samples, as described in Colombi & Walter (2016). During wheat growth, the oxygen concentration in soil air was monitored in four additional soil columns of each soil treatment via 4 cm long gas-permeable tubes connected to gastight access tubes inserted to 6 cm depth in the bulk soil. Oxygen concentration in soil air was measured daily from

3 to 18 DAS with a commercial gas analyser (CheckMate 9900 benchtop headspace O₂/CO₂ gas analyser; PBI Dansensor A/S, Ringsted, Denmark) in the morning immediately after watering.

In the wheat plots, three undisturbed soil samples of 100 ml and 5 cm height per block were taken from 10 and 30 cm depth in each treatment. Gas transport characteristics and mechanical impedance were measured as described above, at a matric potential of -100 hPa. Soil bulk density was determined from the same field samples using the oven-drying method (105°C for four days). Soil bulk density was also measured in the soybean plots (only Com and Ctrl) and the maize plots (all treatments) at 10 and 30 cm depth, with three replicates per block.

2.4. Interaction of roots with artificial pores

The interaction of crop roots with inserted macropores was quantified from X-ray micro CT scans (Phoenix v|tome|x s 240; GE Sensing and Inspection Technologies GmbH, Wunstorf, Germany). For plants from the growth chamber, the entire soil column was scanned using the multi-scan option. In the field, two undisturbed samples of 10 cm diameter and 10 cm height were excavated in PVC tubes from 0-10 cm and 13-23 cm depth of each perforated square (Figure 1c). The excavated samples were kept moist and stored at 3°C until scanning. The settings used resulted in a 68 µm and 120 µm voxel edge length for soil columns and field samples, respectively (Supplementary data Table S2). Visual detection limits for structures in X-ray CT scans are at diameters that exceed twice the voxels edge length (Jähne, 2002). Hence the visual detection limit for roots was at a diameter of around 170 µm and 300 µm for samples from the growth chamber and the field, respectively. This represented 2.5 times the respective voxel edge length.

All quantifications from reconstructed CT scans were performed in Visual Studio Max 2.2 (Volume Graphics GmbH, Heidelberg, Germany). In a first step, artificial pores were counted and the number of hits of each pore by roots was determined. To be considered an actual hit, the root needed to fully enter the artificial macropore and not just touch the outer edge. These hits were then divided into two different classes of interaction: ‘crossings’, defined as when the root stayed in the pore for a distance

of less than 5 mm, and ‘colonisations’, defined as when the root grew for a distance of more than 5 mm within the pore (Figure 1d and 1e). In a second step, all visible roots were counted and classified into roots that hit an artificial macropore and roots that never crossed or colonised an artificial macropore. In the case of field samples, statistical evaluation was based on average values for the two soil depths.

2.5. Preferential root growth towards artificial macropores

The observed number of root hits per artificial macropores was compared against the expected number of hits if the roots had grown randomly, which was determined by counting the number of hits per virtually reallocated macropore. For this, artificial pores were segmented using the region-growing algorithm of Visual Studio Max and rotated around the central vertical axis (soil columns 45°, field samples 90°) of the reconstructed scan (Figure 1f). As described above, hits were only counted if the root crossed the virtually reallocated pore and not if it just touched the edge. Expected and observed number of hits were compared in order to determine whether roots grew randomly or grew preferentially towards pores. To further test the hypothesis of preferential root growth, four additional soil columns per crop were prepared. These columns were packed to a bulk density of 1.6 g cm⁻³ and macropores were inserted not in a cross-like pattern, but all in one half of the soil column. To determine the expected number of hits, segmented pores were rotated 180° around the central vertical axis and the observed number of hits was determined as described above.

2.6. Plant vigour

In addition to the field samples from ComAP, four field samples of 10 cm diameter and 10 cm height were taken per block from 0-10 cm and 13-23 cm depth in Ctrl and Com. Roots from all field samples and soil columns were washed gently over a 1 mm sieve and dried at 60°C for four days before weighing.

Just after root samples were excavated from the field, aboveground plant traits were determined using the same plants from which the roots were sampled. In soybean and maize, each belowground sample corresponded to a single plant, whereas in wheat each belowground sample corresponded to 5-8 individual plants. Shoots of plants grown under controlled conditions were sampled 20 DAS immediately after the root system was scanned by X-ray CT. To determine leaf area, leaves of different positions on the stem were harvested, placed on a blue background and covered with a transparent plastic sheet. Together with a size reference, images of leaves were captured under constant illumination with an 18 megapixel camera (Canon EOS 600D; Canon, Tokyo, Japan) with a lens (EF-S18-55mm f/3.5-5.6 ISII; Canon, Tokyo, Japan) fixed to a focal length of 35 mm and an aperture value of 9, resulting in pixel edge length of 60-80 μm . In order to relate the effect of soil treatments to plant development stages, images were taken separately for each leaf level of the main shoot. Leaf area was evaluated in ImageJ version 1.49g (National Institute of Health, Bethesda, MD, United States). Afterwards, entire shoots were dried at 60°C for four days prior to weighing.

2.7. Statistical evaluation

Chi-square tests were used to compare observed and expected number of root hits per artificial macropores. All other evaluations were based on analysis of variance (ANOVA) and treatment means were compared using ANOVA least significant (LSD) tests. For air permeability, the raw data were converted to base 10 logarithm before statistical evaluation. All statistical analyses were performed in R version 3.1.3 (R Development Core Team, Vienna, Austria).

3. Results

3.1. Soil physical properties

Soil physical responses to soil compaction and to subsequent perforation of compacted soil were highly consistent between packed soil from the growth chamber and undisturbed soil from the field experiments. Mechanical impedance in packed and undisturbed soil samples was increased by 20-200% due to soil compaction and not affected by artificial macropores in compacted soil (Table 1). However, the artificial macropores created possible pathways of very low or no mechanical impedance. Air permeability (K_a) and relative gas diffusion coefficient (D_p/D_0) decreased as a result of compaction. Creation of artificial macropores in compacted soil significantly increased gas transport rates in undisturbed and packed soil samples. The D_p/D_0 and $\log_{10}(K_a)$ values obtained were at least 1.5-fold higher in compacted soil with artificial macropores than in compacted soil without artificial macropores (Table 1). Furthermore, oxygen concentrations in soil air measured during wheat growth under controlled conditions differed between soil treatments. Measured oxygen concentrations in bulk soil air were highest in uncompacted soil, followed by compacted soil with artificial macropores. The lowest oxygen concentrations were recorded in the air from compacted soil without artificial macropores (Supplementary data Fig. S1). Soil bulk density in the field was increased by soil compaction, particularly at 10 cm depth. Artificial macropores in compacted soil did not affect soil bulk density compared with that in compacted soil without artificial macropores, except at 10 cm depth in the wheat plots (Table 2). The difference in bulk density between ComAP and Com in that case was possibly caused by the long time span of 218 days between soil perforation and sampling.

3.2. Interaction of roots with artificial pores

For all three crop species studied, between 0.74 and 1.65 root hits per single artificial macropore were counted in the CT scans from the growth chamber and the field. Once reaching an artificial macropore, the roots of soybean, wheat and maize behaved differently. Under both controlled and field conditions,

the interactions of maize roots with artificial pores were predominantly characterised as colonisations, which occurred 54-122% more frequently than crossings (Figure 2). In contrast to maize, soybean roots crossed and colonised artificial macropores to an equal extent (Figure 2). Due to sample size and the resulting visual detection limit for roots in the reconstructed CT scans, fine roots of soybean could not be quantified in the field samples. This resulted in a lower number of observed hits in field samples compared with samples from the growth chamber (Figure 2, Table 3). Also due to different voxel resolutions lateral roots of wheat could be seen in scans from the growth chamber, but not in scans from the field. Under controlled conditions, interactions of wheat roots with artificial macropores were predominantly classified as crossings (Figure 2). In the field, colonisations of artificial pores by wheat roots occurred slightly more often than crossings (Figure 2). However, the number of crossings in the field was probably underestimated, since lateral roots, which grow more horizontally than main roots, were not visible in the scans.

Although root-pore interactions were quantified in all three species, most of the roots in the samples did not interact with the artificial macropores. Between 72 and 91% of the roots visible in the reconstructed CT scans neither crossed nor colonised artificial macropores (Figure 3).

3.3. Preferential root growth towards artificial macropores

Virtually reallocating the artificial macropores within the reconstructed scan (Figure 1f) allowed the expected number of hits per pore if roots had grown randomly to be estimated. For all three crops and in both environments, the expected number of hits was between 36% and 64% lower than the observed number when pores were at their actual position. This was particularly obvious in the soil columns where the artificial macropores were limited to one half of the column. Chi-square tests were highly significant for all crop-environment combinations (p-values between 0.025 and <0.001; Table 3).

Based on these findings, the quantified interaction of roots with artificial macropores was not a coincidence, but was the result of directed root growth towards the pores. This directed growth was also evident when using a rather qualitative and visual approach, where bending of roots towards a nearby artificial macropore was observed (Figure 4a). Furthermore, in the treatment where macropores

were inserted into only one half of the soil columns, more roots were present in the perforated half than in the unperforated half and roots in the perforated half grew deeper (Figure 4b and c).

3.4. Plant vigour

Significant differences in vigour traits of 20-day-old seedlings from the growth chamber were observed between densely and loosely packed soil columns. Artificial macropores increased plant vigour compared with plants from compacted columns, but the differences were not significant (Supplementary data Table S3). Root dry weight of all crops decreased by 36-72% in Com and ComAP compared with Ctrl. The reduction in shoot dry weight was of a similar order of magnitude for wheat and maize, whereas for soybean the decrease in shoot dry weight was not significant. Total leaf area was reduced by 26-70% in the ComAP and Com treatments compared with loosely packed columns. Root-shoot ratio of all species was not affected by different soil treatments (Supplementary data Table S3).

In the field, all three species showed decreased plant vigour due to soil compaction, but also significantly increased vigour due to the presence of artificial macropores in the compacted soil. Comparison of leaf area for different leaf positions on the stem showed that the beneficial effects of artificial macropores in compacted soil on plant growth occurred later than the adverse effects of compaction. In soybean, leaf area decreased in Com and ComAP compared with Ctrl starting at the 6th leaf. Leaf area from the 7th to the 10th leaf was larger in ComAP than in Com (Figure 5). In maize, the observed pattern of leaf area with respect to leaf position was very similar to that in soybean. Adverse effects of soil compaction on plant vigour were visible at the 4th leaf, while from the 8th leaf onwards, leaf area increased under ComAP compared with Com (Figure 5). The effects of the different soil treatments on the productivity of winter wheat were slightly different. Leaf area at individual leaf positions reflected the effect of soil compaction, but not of artificial pores in compacted soil, on plant vigour development. However, compared with wheat plants grown on compacted soil, plants from compacted plots with artificial macropores developed significantly more tillers (Figure 5). Shoot dry weight also responded significantly to soil compaction and the presence of artificial macropores in

compacted soil. On uncompacted and compacted soil with artificial macropores, the mean dry weight of individual soybean plants was around 22 g, compared with 15 g under compaction without artificial macropores. In wheat, the effects of soil treatments on plant dry weight were of a similar order of magnitude: Shoot dry weight dropped from around 6 g per plant in Ctrl and ComAP to 3.6 g per plant in Com (Figure 5). Due to strong block effects, significant differences in plant dry weight were only found between Ctrl and Com (Supplementary data Table S4). Dry weight of single plants decreased from 131 g in the uncompacted plots to 107 g and 85 g under ComAP and Com, respectively (Figure 5).

Root dry weight, expressed as the sum of root dry matter in both sampled depths, was also reduced due to soil compaction. In soybean, wheat and maize, root dry weight was decreased under compaction by roughly 40% compared with the uncompacted plots. Root dry weight in compacted plots with artificial macropores was around 40% higher than in compacted plots for wheat and soybean, whereas in maize just a very small increase in root dry weight was observed due to macropores in compacted soil (Supplementary data Table S3).

4. Discussion

Quantification of the interactions between roots and artificial macropores in compacted soil revealed that roots of the three crop species studied grew preferentially towards spots with more favourable soil physical conditions. The X-ray micro CT scans showed that roots of different species interacted differently with artificial macropores created in compacted soil. By combining experiments in a growth chamber with field experiments, it was possible to show that these responses were consistent between controlled and field conditions. The data also showed that soil compaction decreased plant growth at an early developmental stage, whereas artificial pores in compacted soil enabled the plant to compensate for the decreased early vigour at later developmental stages.

4.1. Artificial macropores increased gas transport rates in compacted soil

Soil physical properties differed significantly between loose, compacted and compacted soil with artificial macropores. As proposed previously (Colombi and Walter, 2016; Stirzaker et al., 1996), the combination of different soil physical measurements provided an adequate picture of the physical environment of roots. In particular, the measurements of soil physical functions showed the effects of the different soil treatments on soil physical fertility. The highest gas diffusivity and air permeability values were recorded in ComAP, followed by Ctrl and Com (Table 1). Furthermore, the oxygen concentration in soil air measured in soil columns was increased in compacted bulk soil due to the presence of nearby artificial macropores (Supplementary data Fig. S1). Particularly in poorly aerated soil such as compacted soil and under wet conditions, macropores can mediate soil hypoxia. These observations confirm findings in previous studies regarding the effects of macropores on soil physical fertility. Naturally occurring and experimentally constructed macropores increase fluid transport rates and were described as potential pathways for roots to reach water and nutrients (Kuncoro et al., 2014b; Stirzaker et al., 1996; Valentine et al., 2012). Mechanical impedance in the bulk soil was only affected by soil compaction and not by the presence of artificial macropores (Table 1). Soil bulk density in the

field was generally not different between Com and ComAP, except at 10 cm depth in the wheat plots (Table 2). Uplift of soil caused by increased root growth (Supplementary data Table S3) or more pronounced wetting-drying cycles due to the presence of artificial macropores may have caused this discrepancy. Nevertheless, these results show that certain soil physical functions that are adversely affected by compaction can be recovered by inserting continuous vertical macropores into compacted soil.

4.2. Roots of different species interacted differently with artificial macropores

In the present study roots of soybean, wheat and maize showed differing behaviour towards cylindrical and vertical artificial macropores, which provided a potential path of least resistance and a continuous connection to atmospheric air. In the case of maize, the artificial pores were mainly used as a path of least resistance, since most interactions were classified as colonisations (Figure 2). Such root behaviour in searching for and following a pore, as a path of least resistance, was described as ‘trematotropism’ by Dexter (1986). Trematotropism was also observed to some extent for soybean roots and to a lesser extent for wheat roots. In soybean, less than 50% of the roots that reached a pore remained within it. The remaining soybean roots interacting with artificial pores crossed the pores (Figure 2). In contrast to maize, wheat roots predominantly crossed artificial pores rather than remaining within them (Figure 2). This behaviour was particularly observed in scans from the growth chamber samples, where lateral roots were clearly visible. Owing to their horizontal growth direction, lateral roots are more likely to re-enter the bulk soil immediately after entering an artificial macropore than vertically growing main roots. Furthermore, it has been shown that lateral roots preferentially grow into less porous areas in the soil (Bao et al., 2014). The number of crossings in field samples may have been underestimated, however, since lateral roots of wheat were not visible due to the lower voxel resolution. Overall, the observed trematotropic behaviour of crop roots is in line with other reports of artificial and natural macropores being used by roots as a path of least resistance to reach deeper soil layers (Athmann et al., 2013; de Freitas et al., 1999; Dexter, 1986; Han et al., 2015; Kautz et al., 2013; Nakamoto, 1997; Stirzaker et al., 1996; White and Kirkegaard, 2010).

The methodologies used in previous studies, such as endoscopy and examinations of profile walls or slices of soil cores, did not allow crossings of macropores by roots as defined in the present study to be observed. Use of X-ray CT in a previous pot study revealed that barley roots also cross, rather than colonise, artificial macropores in compacted soil (Pfeifer et al., 2014b). The observed crossings of artificial macropores by roots indicate that roots used the macropores as a source for oxygen, which is needed to maintain aerobic root respiration. This effect would be particularly pronounced under moist and poorly aerated conditions (Dexter, 1986). Continuous and vertical macropores accelerate soil drainage (Chen et al., 2014; Kuncoro et al., 2014a, 2014b; Lipiec and Hatano, 2003) and increase oxygen supply to the soil (Supplementary data Figure S1). Based on the results of the present study, there are two possible explanations for the differing behaviour of roots of different plant species to macropores. On the one hand, the difference between the species could be explained by their tolerance to increased mechanical impedance. Maize, which predominantly used macropores as a path of least resistance (Figure 2), is known for its relatively low tolerance to increased mechanical impedance compared with small-grain cereals (Grzesiak et al., 2014). Wheat, in contrast, predominantly crossed macropores (Figure 2) and is known to be less sensitive to increased impedance than maize (Grzesiak et al., 2014). The tolerance of soybean roots to increased mechanical impedance was observed to be higher than that of maize (Bushamuka and Zobel, 1998), but lower than that of wheat (Busscher et al., 2000). This corresponds to our finding that soybean roots were equally likely to cross or colonise an artificial macropore. Besides the different degree of tolerance to mechanical impedance among the investigated species, soil physical properties are suggested to determine as well whether roots remain in artificial macropores or only cross them. The likelihood that roots would stay in the pores presumably increases when the mechanical impedance of the bulk soil increases due to soil drying. Therefore it is also suggested that roots would rather cross such vertical macropores under moist conditions, when re-entering into the bulk soil is less restricted by mechanical impedance.

A second possible explanation for the distinctly different behaviour between roots of different species with artificial macropores is root-soil contact, which is crucial for uptake of e.g. phosphorus or potassium (Stirzaker et al., 1996; Tracy et al., 2011). It has been observed previously that macropores are often occupied by more than one living or dead root (White and Kirkegaard, 2010), which enables

better root-soil contact than with a single root. In the present study, the artificial pores made in compacted soil were 1.25 mm in diameter and the diameter of maize roots is usually larger than 1 mm (Lipiec et al., 2012), enabling good root soil contact within the artificial macropores. Wheat and newly emerging soybean roots have smaller diameter (Colombi and Walter, 2016; Lipiec et al., 2012; Tracy et al., 2012), which would not allow for sufficient root-soil contact within artificial pores.

4.3. Roots grew preferentially towards artificial macropores

Despite the differences in interaction with artificial macropores between roots of wheat, soybean and maize, all three species showed directed root growth towards artificial macropores. The observed number of hits (sum of crossing and colonising roots per artificial pore) of roots on the artificial pores was 57-178% higher than the number which could be expected if the roots had grown randomly (Table 3, Figure 4). Since the roots needed to grow through compacted bulk soil to reach an artificial macropore, trematotropism alone does not serve as an explanation for such directed growth.

Oxytropism, a phenomena describing the movement or growth towards higher oxygen concentration, has been observed for pea roots (Porterfield and Musgrave, 1998). Taking into account the increased gas diffusivity and air permeability due to artificial macropores in the present study (Table 1, Supplementary data Fig. S1), oxytropism seems to be the driving mechanism for roots to sense pores and grow towards them. It has been suggested that roots sense different environmental properties and prefer to grow into more favourable parts of the soil and avoid more hostile parts (Passioura, 2002). In our study, artificial macropores and the bulk soil immediately beside these represented such spots of favourable soil physical conditions. Moreover, they provided roots with an environment in and around which soil physical functions had at least partially recovered from compaction. Oxygen transport to the bulk soil around the artificial pores was accelerated (Supplementary data Fig. S1) and the artificial macropores served as a continuous connection to atmospheric air and as a path of least resistance. A few other studies have observed similar results, such as increased lateral root initiation into wetter soil (Bao et al., 2014) and avoidance of unfavourable soil structure (Semchenko et al., 2008). Attraction of roots to macropores has been proposed (Pfeifer et al., 2014b; Stirzaker et al., 1996; White and

Kirkegaard, 2010), but to the best of our knowledge has not been quantified previously. As reported in other studies (Cornish, 1993; Han et al., 2015; Pfeifer et al., 2014b; Rasse and Smucker, 1998), the vast majority (>70%) of the roots neither crossed nor colonised artificial macropores (Figure 3).

4.4. Artificial macropores in compacted soil compensated for decreased early vigour at later developmental stages

Plant vigour of wheat, soybean and maize was significantly altered by the different soil treatments. Compared with the uncompacted treatment, dry shoot biomass of all three crop species was decreased due to compaction, by 24-70% and 31-45% in the columns and the field, respectively (Figure 5, Supplementary data Table S3). Dry root biomass in the samples excavated from the field and the soil columns decreased in a similar order of magnitude in response to compaction (Supplementary data Table S3). Decreasing crop productivity resulting from restricted access to deeper soil layers (McKenzie et al., 2009) and soil compaction has been shown for various species in the field (Arvidsson and Håkansson, 2014; Barraclough and Weir, 1988; Botta et al., 2010; Colombi and Walter, 2016). Also in young plants, grown under laboratory conditions soil compaction results in reduced plant vigour (Bingham and Bengough, 2003; Buttery et al., 1998; de Freitas et al., 1999; Grzesiak et al., 2013; Young et al., 1997). Such studies with seedlings have shown that the adverse effects of soil compaction on plant growth occur at very early developmental stages, which corresponds to the findings in this study. In response to soil compaction, dry shoot biomass of plants from the growth chamber in the present study was decreased at 20 DAS (Supplementary data Table S3). Plants grown in compacted soil in the field showed progressively decreasing leaf area starting between leaf position 4-6 compared with plants from uncompacted soil (Figure 5). Comparing the experimental data for young and mature plants showed that the beneficial effects of artificial macropores in compacted soil on plant vigour occurred later than the adverse effects of compaction. In the growth chamber, plants were too young to show increased vigour due to artificial macropores in compacted soil (Supplementary data Table S3). In the field, leaf area increased from leaf position 7 and 9 onwards in soybean and maize, respectively, in ComAP compared with Com. In wheat, this

effect occurred during tillering stage (Figure 5). As previously observed in laboratory studies (de Freitas et al., 1999; Stirzaker et al., 1996), dry shoot biomass in the field was 27-67% higher due to the artificial pores in compacted soil (Figure 5). Remarkably, this increase in biomass occurred even though less than 30% of the roots crossed or colonised artificial macropores (Figure 4). These findings suggest that in an early stage of plant development, the increased mechanical impedance of compacted bulk soil led to decreased early vigour. As plant development progressed, soil functions provided specifically by artificial macropores in compacted soil, allowed the plant to compensate for poor early vigour. Since crossing and colonisation occurred in all three studied species, the artificial macropores in compacted soil fulfilled different purposes for the growing plant. Increased soil aeration due to the artificial macropores helped to maintain aerobic root respiration, whereas roots used artificial macropores as a path of least resistance to reach resources in deeper soil layers. This combination of providing oxygen and a potential path of least resistance for growing roots was most likely the cause for the beneficial effects of artificial macropores in compacted soil on plant vigour.

5. Conclusions

Combining soil physical measurements with observations of root-pore interactions and detailed shoot measurements allowed the influence of different soil physical conditions on belowground and aboveground plant development to be quantified. The results showed that roots sense their physical environment and grow preferentially towards artificial macropores. The benefit of vertical macropores for plant growth is evident when considering the increased soil aeration due to perforation of compacted soil (Table 1) and the different interactions of roots with these pores (Figure 2). Besides serving as a path of least resistance to reach deeper soil strata, macropores served as a source of oxygen for growing roots. Thus creation of vertical macropores increased soil physical fertility and resulted in increased crop productivity of all three species. For future research it would be desirable to investigate root-macropore interactions and directed root growth towards macropores at a range of soil physical conditions and to look at the underlying mechanisms of preferential growth. Since macropores in naturally structured soil cover a wide range of shapes, geometries and orientations, this should additionally be taken into account in further studies on root-macropore interactions.

Acknowledgements

The authors express their gratitude to Marlies Sommer and Dr. Jan Rek from Agroscope Zurich, Switzerland, for their assistance measuring fluid transport rates. Siul Ruiz, Dr. Stan Schymanski, Daniel Breitenstein and Prof. Dani Or (ETH Zürich, Switzerland) are thanked for stimulating discussions and the help with soil penetration test. Thanks also to Laurin Müller (ETH Zürich, Switzerland) and Rodolfo Colombi for their help in the field and Patrick Flütsch (ETH Zürich, Switzerland) for technical support. Funding for the study was provided by the Swiss National Science Foundation (project no 406840-143061) within the framework of the national research programme 68 (Soil as a Resource, www.nrp68.ch).

References

- Abbott, L.K., Murphy, D. V, 2007. Soil Biological Fertility. A key to sustainable Land Use in Agriculture. Springer Netherlands.
- Alameda, D., Villar, R., 2012. Linking root traits to plant physiology and growth in *Fraxinus angustifolia* Vahl. seedlings under soil compaction conditions. *Environ. Exp. Bot.* 79, 49–57. doi:10.1016/j.envexpbot.2012.01.004
- Arvidsson, J., Håkansson, I., 2014. Response of different crops to soil compaction—Short-term effects in Swedish field experiments. *Soil Tillage Res.* 138, 56–63. doi:10.1016/j.still.2013.12.006
- Athmann, M., Kautz, T., Pude, R., Köpke, U., 2013. Root growth in biopores—evaluation with in situ endoscopy. *Plant Soil* 371, 179–190. doi:10.1007/s11104-013-1673-5
- Bao, Y., Aggarwal, P., Robbins, N.E., Sturrock, C.J., Thompson, M.C., Tan, H.Q., Tham, C., Duan, L., Rodriguez, P.L., Vernoux, T., Mooney, S.J., Bennett, M.J., Dinneny, J.R., 2014. Plant roots use a patterning mechanism to position lateral root branches toward available water. *Proc. Natl. Acad. Sci. U. S. A.* 111, 9319–24. doi:10.1073/pnas.1400966111
- Barracough, P.B., Weir, A.H., 1988. Effects of a compacted subsoil layer on root and shoot growth, water use and nutrient uptake of winter wheat. *J. Agric. Sci.* 110, 207–216. doi:10.1017/S0021859600081235
- Batey, T., 2009. Soil compaction and soil management - a review. *Soil Use Manag.* 25, 335–345. doi:10.1111/j.1475-2743.2009.00236.x
- Bingham, I.J., Bengough, A.G., 2003. Morphological plasticity of wheat and barley roots in response to spatial variation in soil strength. *Plant Soil* 250, 273–282.
- Botta, G.F., Tolon-Becerra, a., Lastra-Bravo, X., Tourn, M., 2010. Tillage and traffic effects (planters and tractors) on soil compaction and soybean (*Glycine max* L.) yields in Argentinean pampas. *Soil Tillage Res.* 110, 167–174. doi:10.1016/j.still.2010.07.001
- Bottinelli, N., Hallaire, V., Goutal, N., Bonnaud, P., Ranger, J., 2014. Impact of heavy traffic on soil macroporosity of two silty forest soils: Initial effect and short-term recovery. *Geoderma* 217-218,

- Bushamuka, V.N., Zobel, R.W., 1998. Differential genotypic and root type penetration of compacted soil layers. *Crop Sci.* 38, 776–781.
- Busscher, W.J., Frederick, J.R., Bauer, P.J., 2000. Timing effects of deep tillage on penetration resistance and wheat and soybean yield. *Soil Sci. Soc. Am. J.* 64, 999–1003.
- Buttery, B.R., Tan, C.S., Drury, C.F., Park, S.J., Armstrong, R.J., Park, K.Y., 1998. The effects of soil compaction, soil moisture and soil type on growth and nodulation of soybean and common bean. *Can. J. Plant Sci.* 571–576.
- Chen, G., Weil, R.R., Hill, R.L., 2014. Effects of compaction and cover crops on soil least limiting water range and air permeability. *Soil Tillage Res.* 136, 61–69. doi:10.1016/j.still.2013.09.004
- Coelho Filho, M. a., Colebrook, E.H., Lloyd, D.P. a., Webster, C.P., Mooney, S.J., Phillips, A.L., Hedden, P., Whalley, W.R., 2013. The involvement of gibberellin signalling in the effect of soil resistance to root penetration on leaf elongation and tiller number in wheat. *Plant Soil* 371, 81–94. doi:10.1007/s11104-013-1662-8
- Colombi, T., Walter, A., 2016. Root responses of triticale and soybean to soil compaction in the field are reproducible under controlled conditions. *Funct. Plant Biol.* 43, 114–128. doi:dx.doi.org/10.1071/FP15194
- Cornish, P.S., 1993. Soil macrostructure and root growth of establishing seedlings. *Plant Soil* 151, 119–126. doi:10.1007/BF00010792
- de Freitas, P.L., Zobel, R.W., Snyder, V.A., 1999. Corn Root Growth in Soil Columns with Artificially Constructed Aggregates. *Crop Sci.* 39, 725–730.
- Dexter, A.R., 1991. Amelioration of soil by natural processes. *Soil Tillage Res.* 20, 87–100.
- Dexter, A.R., 1986. Model experiments on the behaviour of roots at the interface between a tilled seed-bed and a compacted sub-soil III. Entry of pea and wheat roots into cylindrical biopores. *Plant Soil* 95, 149–161.
- Downie, H., Holden, N., Otten, W., Spiers, A.J., Valentine, T. a, Dupuy, L.X., 2012. Transparent soil

- for imaging the rhizosphere. PLoS One 7, e44276. doi:10.1371/journal.pone.0044276
- Grzesiak, M.T., Ostrowska, A., Hura, K., Rut, G., Janowiak, F., Rzepka, A., Hura, T., Grzesiak, S., 2014. Interspecific differences in root architecture among maize and triticale genotypes grown under drought, waterlogging and soil compaction. *Acta Physiol. Plant.* 36, 3249–3261. doi:10.1007/s11738-014-1691-9
- Grzesiak, S., Grzesiak, M.T., Hura, T., Marcińska, I., Rzepka, A., 2013. Changes in root system structure, leaf water potential and gas exchange of maize and triticale seedlings affected by soil compaction. *Environ. Exp. Bot.* 88, 2–10. doi:10.1016/j.envexpbot.2012.01.010
- Hamza, M. a., Anderson, W.K., 2005. Soil compaction in cropping systems. *Soil Tillage Res.* 82, 121–145. doi:10.1016/j.still.2004.08.009
- Han, E., Kautz, T., Perkons, U., Uteau, D., 2015. Root growth dynamics inside and outside of soil biopores as affected by crop sequence determined with the profile wall method. *Biol. Fertil. ...* 51, 847–856. doi:10.1007/s00374-015-1032-1
- Hernandez-Ramirez, G., Lawrence-Smith, E.J., Sinton, S.M., Tabley, F., Schwen, A., Beare, M.H., Brown, H.E., 2014. Root Responses to Alterations in Macroporosity and Penetrability in a Silt Loam Soil. *Soil Sci. Soc. Am. J.* 78, 1392–1403. doi:10.2136/sssaj2014.01.0005
- Iijima, M., Kato, J., 2007. Combined Soil Physical Stress of Soil Drying , Anaerobiosis and Mechanical Impedance to Seedling Root Growth of Four Crop Species. *Plant Prod. Sci.* 10, 451–459.
- Jähne, B., 2002. *Digital Image Processing*, 5th ed. Springer Berlin Heidelberg, Berlin, Heidelberg. doi:10.1007/978-3-662-04781-1
- Kautz, T., Perkons, U., Athmann, M., Pude, R., Köpke, U., 2013. Barley roots are not constrained to large-sized biopores in the subsoil of a deep Haplic Luvisol. *Biol. Fertil. Soils* 49, 959–963. doi:10.1007/s00374-013-0783-9
- Kuncoro, P.H., Koga, K., Satta, N., Muto, Y., 2014a. A study on the effect of compaction on transport properties of soil gas and water I: Relative gas diffusivity, air permeability, and saturated

- hydraulic conductivity. *Soil Tillage Res.* 143, 172–179. doi:10.1016/j.still.2014.02.006
- Kuncoro, P.H., Koga, K., Satta, N., Muto, Y., 2014b. A study on the effect of compaction on transport properties of soil gas and water. II: Soil pore structure indices. *Soil Tillage Res.* 143, 180–187. doi:10.1016/j.still.2014.01.008
- Lipiec, J., Hatano, R., 2003. Quantification of compaction effects on soil physical properties and crop growth. *Geoderma* 116, 107–136. doi:10.1016/S0016-7061(03)00097-1
- Lipiec, J., Horn, R., Pietrusiewicz, J., Siczek, A., 2012. Effects of soil compaction on root elongation and anatomy of different cereal plant species. *Soil Tillage Res.* 121, 74–81. doi:10.1016/j.still.2012.01.013
- McKenzie, B.M., Bengough, A.G., Hallett, P.D., Thomas, W.T.B., Forster, B., McNicol, J.W., 2009. Deep rooting and drought screening of cereal crops: A novel field-based method and its application. *F. Crop. Res.* 112, 165–171. doi:10.1016/j.fcr.2009.02.012
- Metzner, R., Eggert, A., van Dusschoten, D., Pflugfelder, D., Gerth, S., Schurr, U., Uhlmann, N., Jahnke, S., 2015. Direct comparison of MRI and X-ray CT technologies for 3D imaging of root systems in soil: potential and challenges for root trait quantification. *Plant Methods* 11, 17. doi:10.1186/s13007-015-0060-z
- Nakamoto, T., 1997. The Distribution of Maize Roots as Influenced by Artificial Vertical Macropores. *Japanese J. Crop Sci.* 66, 331–332.
- Passioura, J.B., 2002. Soil conditions and plant growth. *Plant, Cell Environ.* 25, 311–318. doi:10.1046/j.0016-8025.2001.00802.x
- Pfeifer, J., Faget, M., Walter, A., Blossfeld, S., Fiorani, F., Schurr, U., Nagel, K. a., 2014a. Spring barley shows dynamic compensatory root and shoot growth responses when exposed to localised soil compaction and fertilisation. *Funct. Plant Biol.* 41, 581–597. doi:10.1071/FP13224
- Pfeifer, J., Kirchgessner, N., Colombi, T., Walter, A., 2015. Rapid phenotyping of crop root systems in undisturbed field soils using X-ray computed tomography. *Plant Methods* 11, 41. doi:10.1186/s13007-015-0084-4

- Pfeifer, J., Kirchgessner, N., Walter, A., 2014b. Artificial pores attract barley roots and can reduce artifacts of pot experiments. *J. Plant Nutr. Soil Sci.* 177, 903–913. doi:10.1002/jpln.201400142
- Porterfield, D.M., Musgrave, M.E., 1998. The tropic response of plant roots to oxygen: Oxytropism in *Pisum sativum* L. *Planta* 206, 1–6. doi:10.1007/s004250050367
- Rasse, D.P., Smucker, A.J.M., 1998. Root recolonization of previous root channels in corn and alfalfa rotations. *Plant Soil* 204, 203–212. doi:10.1023/A:1004343122448
- Schjonning, P., Rasmussen, K.J., 2000. Soil strength and soil pore characteristics for direct drilled and ploughed soils. *Soil Tillage Res.* 57, 69–82. doi:10.1016/S0167-1987(00)00149-5
- Scholl, P., Leitner, D., Kammerer, G., Loiskandl, W., Kaul, H.-P., Bodner, G., 2014. Root induced changes of effective 1D hydraulic properties in a soil column. *Plant Soil* 381, 193–213. doi:10.1007/s11104-014-2121-x
- Semchenko, M., Zobel, K., Heinemeyer, A., Hutchings, M.J., 2008. Foraging for space and avoidance of physical obstructions by plant roots: A comparative study of grasses from contrasting habitats. *New Phytol.* 179, 1162–1170. doi:10.1111/j.1469-8137.2008.02543.x
- Siczek, A., Lipiec, J., Wielbo, J., Szarlip, P., Kidaj, D., 2013. Pea growth and symbiotic activity response to Nod factors (lipo-chitoooligosaccharides) and soil compaction. *Appl. Soil Ecol.* 72, 181–186. doi:10.1016/j.apsoil.2013.06.012
- Stirzaker, R.J., Passioura, J.B., Wilms, Y., 1996. Soil structure and plant growth: Impact of bulk density and biopores. *Plant Soil* 185, 151–162. doi:10.1007/BF02257571
- Suzuki, L.E.A.S., Reichert, J.M., Reinert, D.J., 2013. Degree of compactness, soil physical properties and yield of soybean in six soils under no-tillage. *Soil Res.* 51, 311–321. doi:10.1071/SR12306
- Tracy, S.R., Black, C.R., Roberts, J. a, Mooney, S.J., 2011. Soil compaction: a review of past and present techniques for investigating effects on root growth. *J. Sci. Food Agric.* 91, 1528–37. doi:10.1002/jsfa.4424
- Tracy, S.R., Black, C.R., Roberts, J. a, McNeill, A., Davidson, R., Tester, M., Samec, M., Korošak, D., Sturrock, C., Mooney, S.J., 2012. Quantifying the effect of soil compaction on three varieties

- of wheat (*Triticum aestivum* L.) using X-ray Micro Computed Tomography (CT). *Plant Soil* 353, 195–208. doi:10.1007/s11104-011-1022-5
- Valentine, T. a, Hallett, P.D., Binnie, K., Young, M.W., Squire, G.R., Hawes, C., Bengough, a G., 2012. Soil strength and macropore volume limit root elongation rates in many UK agricultural soils. *Ann. Bot.* 110, 259–70. doi:10.1093/aob/mcs118
- Van den Akker, J.J.H., Canarache, A., 2001. Two European concerted actions on subsoil compaction. *L. Use Dev.* 42, 15–22.
- Walter, A., Silk, W.K., Schurr, U., 2009. Environmental effects on spatial and temporal patterns of leaf and root growth. *Annu. Rev. Plant Biol.* 60, 279–304. doi:10.1146/annurev.arplant.59.032607.092819
- White, R.G., Kirkegaard, J.A., 2010. The distribution and abundance of wheat roots in a dense, structured subsoil – implications for water uptake. *Plant. Cell Environ.* 33, 133–148. doi:10.1111/j.1365-3040.2009.02059.x
- Young, I.M., Montagu, K., Conroy, J., Bengough, A.G., 1997. Mechanical impedance of root growth directly reduces leaf elongation rates of cereals. *New Phytol.* 135, 613–619. doi:10.1046/j.1469-8137.1997.00693.x

Table 1: Mechanical impedance, air permeability (Ka) and relative gas diffusion coefficients (Dp/D0) of uncompacted (Ctrl), compacted (Com) and compacted soil with artificial macropores (ComAP) measured in packed (soil columns) and undisturbed (field) soil cores at a matric potential of -100 hPa. Different letters indicate significant differences (p<0.05) of the means using least significant difference (LSD) test (soil columns n=4, field n=3).

Environment	Parameter	Ctrl	Com	ComAP	LSD
Soil columns	Impedance [MPa]	0.34 ^a	1.06 ^b	0.98 ^b	0.26
	log ₁₀ (Ka [μm ²])	0.32 ^a	0.04 ^a	1.40 ^b	0.62
	Dp/D0 [-]	0.0008 ^a	0.0009 ^a	0.0027 ^b	0.0017
Field 10 cm depth	Impedance [MPa]	0.81	1.09	0.95	0.42
	log ₁₀ (Ka [μm ²])	0.60 ^{ab}	0.38 ^a	1.11 ^b	0.53
	Dp/D0 [-]	0.0040	0.0013	0.0089	0.0079
Field 30 cm depth	Impedance [MPa]	0.56 ^a	0.86 ^b	1.08 ^b	0.25
	log ₁₀ (Ka [μm ²])	0.65 ^{ab}	0.38 ^a	1.05 ^b	0.59
	Dp/D0 [-]	0.0036 ^{ab}	0.0002 ^a	0.0064 ^b	0.0060

Table 2: Soil bulk density (ρ_b) in the field in uncompacted (Ctrl), compacted (Com) and compacted soil with artificial macropores (ComAP) for the three crop species tested. Different letters indicate significant differences ($p < 0.05$) of the means using least significant difference (LSD) test ($n=3$).

Year	Crop	Depth [cm]	ρ_b [g cm ⁻³]:	ρ_b [g cm ⁻³]:	ρ_b [g cm ⁻³]:	LSD
			Ctrl	Com	ComAP	
2014	Soybean	10	1.34 ^a	1.46 ^b	NA	0.09
		30	1.50	1.55	NA	0.16
2015	Wheat	10	1.50 ^{ab}	1.55 ^a	1.43 ^b	0.08
		30	1.44	1.49	1.50	0.11
	Maize	10	1.45 ^a	1.61 ^b	1.59 ^b	0.12
		30	1.40	1.46	1.48	0.10

Table 3: Observed root hits per artificial macropores at their actual position and expected hits of reallocated artificial macropores by roots under controlled conditions in soil columns and the field. Artificial pores were inserted into the soil columns either in a cross-like pattern (Cross) or only in one half of the column (Split), (soil columns n=4, field n=3; chi-square test).

Environment	Pattern	Crop	Observed hits [# pore ⁻¹]	Expected hits [# pore ⁻¹]	p-value
Soil columns	Cross	Soybean	1.65	1.05	0.025
		Wheat	1.65	0.70	<0.001
		Maize	1.30	0.75	<0.001
	Split	Soybean	1.2	0.45	<0.001
		Wheat	1.65	0.65	<0.001
		Maize	1.2	0.65	<0.001
Field		Soybean	0.75	0.39	<0.001
		Wheat	0.74	0.40	<0.001
		Maize	1.28	0.46	<0.001

Figure 1: Perforation of compacted soil with steel wire along a 2 by 2 cm grid in a) soil columns and b) in the field. c) Root sampling in the field in 0-10 cm depth. d) Crossing (Cr) and colonisation (Col) of artificial macropores by soybean roots grown in soil columns. e) Vertical cross-sections of reconstructed computed tomography scans of crossing and colonising soybean roots grown in soil columns. f) Artificial macropores of a field sample at their actual position (grey) and after rotation around the central z-axis (yellow).

2 column fitting image, black-and-white print

Figure 2: Interaction of roots with artificial macropores in compacted soil in a) and b) in soil columns and c) and d) the field, expressed as a) and c) mean number of crossing roots and b) and d) mean number of colonising roots per individual artificial macropore. Different letters indicate significant differences ($p < 0.05$) of the means using the least significant difference (LSD) test. Error bars represent standard error (soil columns $n=4$, field $n=3$).

single column fitting image, black-and-white print

Figure 3: Proportion of total number of counted roots either crossing or colonising (grey) artificial pores or neither crossing or colonising (black) artificial pores in a) soil columns and b) the field. c) Typical picture of maize root system (orange) and artificial macropores (grey transparent) from 0 to 10 cm depth in the field. Error bars represent standard error (soil columns $n=4$, field $n=3$).

single column fitting image, black-and-white print

Figure 4: Illustration of directed root growth towards artificial macropores (grey transparent): a) Soybean main root crossing artificial macropore and lateral root colonising the same artificial macropore. b) maize and c) soybean seedling grown in soil columns: red colour represents roots in compacted soil, green colour roots in perforated compacted soil.

2 column fitting image, black-and-white print

Figure 5: Leaf area with respect to different leaf position for field-grown a) soybean, b) wheat and c) maize plants. Shoot dry weight of field-grown d) soybean, e) wheat and f) maize plants. Shading of all symbols and bars: uncompacted soil (open symbols; Ctrl), compacted soil (closed black symbols; Com) and compacted soil with artificial macropores (closed grey symbols; ComAP). Different letters indicate significant differences ($p < 0.05$) of the means using least significant difference (LSD) test. Error bars represent standard error ($n=3$).

2 column fitting image, black-and-white print