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## Research paper Soil compaction-induced changes of physicochemical properties of cereal roots

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#### ABSTRACT

It is well known, that soil particle surface properties like cation exchange capacity, surface area, and charge density affect ion-exchange reactions in the root zone. However, the exchange processes at the root surface itself also depend on these properties: cation exchange capacity, surface area, which will be determined in this paper for the roots of young wheat, barley, rye, triticale, and maize plants. Undisturbed samples from a Haplic Luvisol developed from loess were taken at 5-10 cm depth from an "normally compacted" core part of the crop field (Ref.) (bulk density 1.29  $\pm$  0.06 Mg m<sup>-3</sup>) and from a "compacted" (C) (bulk density 1.61  $\pm$  0.05 Mg m<sup>-3</sup>) part in the headland. Plants were grown in these soil cores for 11 days in a growth chamber. The negative surface charge of the roots and apparent surface dissociation constants were determined based on potentiometric titration in pH between 3 and 10. The surface charges at pH = 7 and pH = 10 were taken as the root cation exchange capacity (CEC) and total negative surface charge (Qtot), respectively. The differences between the adsorption-desorption data were used to estimate the total apparent surface area (S). The Qtot and S were used to calculate the surface charge density (SCD). Root lengths were determined using WinRHIZO 2007 program. Irrespective of plant species, an increase in the compaction level resulted in an increase in weakly acidic groups and in the total apparent surface area (by 10-25%). On the other hand, strongly acidic groups decreased, as well as the CEC (by 28–45%). Additionally  $Q_{tot}$  (by 19–32%), SCD (by 26–46%), root length (by 12–80%), and shoot mass (by 43-81%) all decreased. The increases in S and decreases in the number of strongly acidic groups, CEC, O<sub>tot</sub>, and SCD in response to soil compaction were less pronounced in coarse-grained maize than in all remaining small-grained cereals. However, the decreases in root length and shoot mass were the highest in maize. The decline in negative surface charge due to soil compaction is attributed to the cell wall structure deformation and lower production and outflow of the acidic groups from the cell interior and lesser quantity of charged root hairs. These results imply the need to consider the root surface charge properties along with root growth traits while studying nutrient uptake from compacted soil.

#### 1. Introduction

The basic chemical soil information includes amongst others the cation exchange capacity (CEC) as well as the surface charge and surface area of the homogenized soil material, which defines the best and/ or complete accessibility of particle surfaces for ion adsorption and desorption. Roots, however, mostly do not have a complete contact with the particle surface and the accessibility of particle surfaces in the soil profile is reduced because of aggregate formation during swelling and shrinkage. In conclusion, cation exchange intensity values are predominantly smaller than 1 if compared with the CEC (Hartmann et al., 1998; Horn et al., 2017). However, in this whole consideration the properties of roots are assumed high enough for ion exchange

although actual data is hardly found in the literature. The surface properties of plant roots need be defined by a root cation exchange capacity (CEC), a total negative surface charge ( $Q_{tot}$ ), apparent surface area (S), and surface charge density (SCD), as they affect the ion binding capacity and ion-exchange reactions in the root zone (Nye and Tinker, 1977; Woodward et al., 1984; Sattelmacher, 2001). It is well known that the binding of cations is a measure of the total negative charges at the root surface and its decrease limits cationic nutrient uptake by plants (Józefaciuk and Szatanik-Kloc, 2003). Therefore, estimates of the roots surface properties are used for modelling cation uptake (Wu and Hendershot, 2009; Ruan et al., 2013) by plants.

CEC and Q<sub>tot</sub> of plant roots result from the presence of a negative charge on the root surface developed from dissociation of acidic

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functional groups present mainly in the cell wall and plasma membrane. The negative charge of the roots is regarded to originate mainly from the carboxyl groups of pectins and hemicelluloses of the cell walls (Carpita and McCann, 2000). However, other cell wall components, such as phenols, proteins, and amino acids, take part in binding and accumulation of cations (Meychik and Yermakov, 2001; Krzesłowska, 2011). The Q<sub>tot</sub> also includes the variable charges due to the phosphate groups of phospholipids and protein-containing amino acids of the plasma membrane (Postma et al., 2005; Kopittke et al., 2011; Wang et al., 2011; Guiges et al., 2014). The root surface charge is finally also correlated with the root apparent surface area (Szatanik-Kloc, 2010).

The root negative charge along with the root apparent surface area consist mainly of cell walls and intercellular spaces (Sattelmacher, 2001) and governs the ratio of cations and uptake thereof by plants (Szatanik-Kloc, 2010). They can be modified by environmental factors and stress conditions (Józefaciuk and Szatanik-Kloc, 2004; Szatanik-Kloc et al., 2009b). It was shown that the CEC and surface charge in roots decreased if aluminium and heavy metals stresses occur or if there is a water deficit (Łukowska and Józefaciuk, 2013; Lukowska and Józefaciuk, 2016). Further, rye roots under zinc stress exhibited a greater specific surface area and a lower total surface charge that led to reduced charge density on the surface (Szatanik-Kloc et al., 2009b).

The response of the surface properties to stress conditions is related with plant species. In a study by Lukowska and Józefaciuk (2016), the decrease in the root CEC in response to osmotic stress was the highest for rye and the lowest for oats. Under aluminium stress, changes in the surface area and adsorption energy of roots were more substantial for the monocot barley than the dicot clover (Szatanik-Kloc et al., 2009a). In a study by Woodward et al. (1984), differences in root CEC between monocot and dicot species and associated dissimilar uptake of monovalent and divalent cations helped explain the differential distribution of grasslands and shrublands and implicated revegetation programs. The changes in the root properties were attributed to damage to the cell walls, a decrease in the quantity of charged root hairs, alterations in the length of roots with a specified diameter, and chemical composition (Silva et al., 2012; Lukowska and Józefaciuk, 2016).

A literature review indicates that most research has been devoted to the response of root surface properties to chemical and water stresses rather than to soil compaction stress although it largely affects root and shoot growth (Hamza and Anderson, 2005; Szatanik-Kloc, 2014, 2016). A characteristic response of roots to soil compaction is decreased root size, thickening, shortening, and lower density of root hairs (Grzesiak et al., 2015), and cell deformation (White and Kirkegaard, 2010; Lipiec et al., 2012). Flattening and elongation of root cells in cereal plants grown in dense soil was accompanied by modifications in the vascular cylinder diameter and cortex thickness (Lipiec et al., 2012). It is, however, mostly unknown what effect has the soil deformation on the exchange properties and functions of roots. In our study the effects of soil compaction on root surface properties were analysed for five cereal species using undisturbed soil samples from compacted and normally compacted areas. The study was inspired by earlier experimental investigations indicating that dense and strong soil substantially changes root morphology and anatomy (White and Kirkegaard, 2010; Lipiec et al., 2012), which can affect the surface properties of roots. We tested the hypothesis that alteration in plant growth conditions due to soil compaction by agricultural machinery affects the root surface properties.

#### 2. Materials and method

#### 2.1. Treatments and growth conditions

The agricultural field was located in Lublin (Poland) ( $51^{\circ} 13' N, 22^{\circ} 37' E$ ) on an Orthic Luvisol derived from loess having clay, silt, and sand content for the topsoil layer (0–20 cm) of 70, 290, and 640 g kg<sup>-1</sup>, respectively, and 11.7 g kg<sup>-1</sup> of organic carbon. Two plots "normally compacted" (Ref.) or "compacted" (C) were taken in the core part of the

field (more than 20 m from the field end) and in the headland (2–6 m from the field end). The C plot vs. the Ref. plot is much more compacted by traffic due to turning around with agricultural implements. The mass of wheel tractors and combine harvesters used varies from 3.5 to 7 Mg and from 7 to 10 Mg, respectively, and the corresponding stress impacts on the soil within the plough layer range from 33 to 76 kPa (Lipiec et al., 2012). Because of their fragile structure, loess soils display low pre-compression stresses (Horn and Fleige, 2003), which are less than the stress induced by machinery and result in plastic (irreversible) deformation. The respective bulk densities under Ref. and C were 1.29 ( $\pm$  0.06) and 1.61 ( $\pm$  0.05) Mg m<sup>-3</sup>.

Undisturbed soil in cylinders with a 50-mm diameter and 200-cm<sup>3</sup> volume was taken from the 5–15 cm depth in spring at soil water content corresponding to field water capacity from both plots. The tested plants included wheat (*Triticum aestivum* cv. Kandela), barley (*Hordeum vulgare* cv. Quench), rye (*Secale cereale* cv. Wisello), triticale (*Triticosecale* Wittmack cv. Cerber), and maize (*Zea mays* cv. Silvano). Seeds were germinated on moist filter paper for 48 h, thereafter, the seedlings were planted at a depth of 8–10 mm into the soil (two seeds per pot). The upper soil layers were covered with a 5-mm layer of perlite to minimise evaporation. Three replicates were used for each plant and compaction level. The experiment was replicated three times.

The cylinders were randomly distributed in a growth chamber (Climatic chamber KK 1200, POL-ECO Aparatura). The plants were grown for 11 days with day-time (16 h) and night-time (8 h) temperatures of 22 °C and 16 °C, respectively, relative humidity of 60%, and photosynthetically active radiation (PAR) of 240–280  $\mu$ mol s<sup>-1</sup> m<sup>-2</sup>. Soil water content corresponding to field water capacity (matric potential – 100 hPa) was maintained at a constant level by weighing the pots and adding water if needed.

At harvest, the shoots of each plant were cut off and the roots were gently washed from the soil and scanned in a film of water (Epson Perfection V700) with a resolution of 600 dpi. The software WinRhizo 2007 (Regent Instruments Inc., Canada) was used to calculate root length for each plant separately. The shoots and roots were dried at room temperature and weighed. Specific root length (SRL) was calculated by dividing root length by dry mass. Then the roots were taken to determine the negative charge by potentiometric titration and the apparent surface area from water vapour desorption isotherms.

#### 2.2. Potentiometric titration

The negative charge of the roots was evaluated by potentiometric titration according to Meychik and Yermakov (2001) with some modifications as described in Szatanik-Kloc et al. (2009b). The titration was performed automatically using the Titrino 702 MS unit (Metrohm AG-Switzerland) in the nitrogen atmosphere. Below we provide a short outline of the method. Root samples of  $0.0500 \pm 0.0001$  g (dry mass) were equilibrated overnight with 20.0 g of a  $1 \text{ mol dm}^{-3}$  sodium chloride (NaCl) solution. Prior to the titration, the pH of every sample was adjusted to 2.95 with 1 mol dm<sup>-3</sup> hydrochloric acid (HCl) and, after 5 min of mixing, the suspension (roots + NaCl) was titrated with 0.1 mol dm<sup>-3</sup> sodium hydroxide (NaOH) in 1 mol dm<sup>-3</sup> NaCl solution at the rate of 0.01 ml min<sup>-1</sup>. When adding the NaOH base (titration), the hydroxyl ions OH<sup>-</sup> neutralize the protons (H<sup>+</sup>) of these groups (e.g.  $COOH + OH^- = COO^- + H_2O$  and produces negatively charged surface anions (e.g. COO<sup>-</sup>) that leads to an increase in a variable surface charge, Q, of the roots. The amount of the titre consumed between pH 3 and 10 was recorded in steps of 0.1 pH unit. The ratio of the amount (moles) of base used for titration of the suspension minus the amount of the titre consumed by the supernatant (NaCl) is equivalent to the variable surface charge of the solid phase (roots),  $\Delta Qv$  (pH). The negative variable surface charge at pH = 7 was taken as the root cation exchange capacity (CEC) according to the method proposed by Morvan et al. (1979), which is based on rinsing plant material with 0.01 mol  $dm^{-3}$  HCl and distilled water and then titration with a solution of KOH or NaOH to

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