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Root and shoot growth of spring wheat (*Triticum aestivum* L.) are differently affected by increasing subsoil biopore density when grown under different subsoil moisture

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Abstract

A column experiment with five different pore densities (0, 1, 2, 3, and 4 pores column^{-1}) and two varying moisture regimes (comparatively dry and comparatively moist regime) in the subsoil part of the columns was established. In each pore, *Lumbricus terrestris* was introduced for 28 days before sowing wheat plants. After 40 days of plant growth, watering was stopped to induce progressive topsoil drying. Parameters describing the shoot hydration, mineral uptake, and aboveground biomass were quantified. Root biomass and root length densities (RLD) were measured separately for six soil layers. Under dry subsoil conditions, plants grown under increasing biopore density showed an increase of the RLD and an improved shoot hydration but the aboveground biomass was unaffected. Since RLD but not root biomass was enhanced, it is assumed that roots were able to explore a larger volume of soil with the same amount of root biomass. Thereby, subsoil water likely was used more efficiently leading to an improved hydration. Under moist subsoil conditions, plants grown with increasing biopore density revealed enhanced shoot biomasses and nutrient uptake while the belowground biomass was unaffected. The improved nutrient uptake can be ascribed to, first, the higher subsoil water availability favoring mass flow driven nutrient uptake, and second, to direct and indirect effects of earthworms on the availability of soil nutrients. It is concluded that high biopore abundancies have the potential to improve not only the belowground but also the aboveground biomass. This, however, largely depends on subsoil moisture.

Keywords Biopore density · Shoot growth · Root length density · Root-shoot ratio · Plant hydration · Nutrient uptake

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Introduction

The subsoil can be an important water source for crops, especially when the topsoil has dried out due to lacking precipitation (Kirkegaard et al. 2007; White and Kirkegaard 2010). This is of great interest as there are several studies indicating that the occurrence of drought periods will increase in Central Europe (Hänsel et al. 2019; Kornhuber et al. 2019). Wheat belongs to the three major cereals besides rice and maize, being one of the most important sources of calories and protein in the world (Daryanto et al. 2016; Zörb et al. 2018). Drought negatively affects the yield performance of wheat in all developmental stages (Abid et al. 2017). During the grain-filling phase, for instance, severe drought stress can cause yield reductions up to 57% (Balla et al. 2011; Zhang et al. 2018). However, drought is threatening not only wheat yields but also those of most crops grown in Central Europe (Hänsel et al. 2019). Thus, strategies to deal with drought are urgently needed. One strategy may be an increased rooting

depth, which has been shown to increase the capture of water under drought conditions from deeper soil layers (Lopes and Reynolds 2010; Lynch and Wojciechowski 2015). However, root growth and the successful access to deeper soil layers is often limited due to both natural and anthropogenic processes (Lynch and Wojciechowski 2015). In agricultural soils, compaction frequently is the result of the use of heavy machinery, for instance tillage machinery (Batey 2009), but can also occur by pedogenesis (Dexter 1986). Biopores have been shown to provide access for plant roots to deeper soil layers and also to bypass compacted soil layers (Stirzaker et al. 1996; Zhou et al. 2020). However, the relevance of biopores as preferential pathways for roots may largely depend on the penetration resistance of soils. Gaiser et al. (2012) have shown that root elongation through biopores increases with bulk density. Accordingly, it is likely that soil moisture influences root growth in biopores since in many soils penetration resistance increases with drought.

Perkons et al. (2014) found that there was an increased root length density of annual field crops in deeper soil layers when they were grown following tap-rooted crops which left biopores in the subsoil. In addition, Gaiser et al. (2012) showed that wheat had a significantly higher water extraction from deeper soil layers when grown following taproot-forming lucerne. Beside taproots, which leave biopores in the soil after their decay, biopores can be formed by soil faunal activity such as the burrowing activity of anecic earthworms. Both taproots and earthworms are leading to more or less round-shaped and void channels with a diameter usually larger than 2 mm (Han et al. 2015; Kautz et al. 2014). Beside a positive effect on the water supply from deeper soil layers, biopores have the potential to improve the nutrient supply of crops, especially if they were formed or inhabited by earthworms. Earthworms are leaving a nutrient enriched drilosphere via coating the pore walls with their excretions (Kautz 2015) or by decomposing and incorporating plant residues from the surface (Mackay and Kladivko 1985). Furthermore, such earthworm burrows often reveal an increased microbial activity and increased mobilization of nutrients (Hoang et al. 2016). Beside the nutrient-enriching effect of earthworms, high biopore abundance can result in increased root length density (RLD). The enhanced root growth through biopores has the potential to increase nutrient uptake from the bulk subsoil (Han et al. 2015). Indeed, Han et al. (2017) demonstrated that barley exhibited a higher shoot K uptake and canola exhibited higher shoot N and P uptakes when grown after the biopore-forming chicory as compared to fibrous rooted tall fescue.

Increased water and nutrient uptake from the subsoil which can be ascribed to the presence of biopores may have the potential to promote not only root but also shoot growth and, finally, crop yield. However, biopores have also been reported to negatively effect on water and nutrient uptake

(Ogilvie et al. 2021). This might be especially true in large-sized biopores (larger than 5 mm diameter). Root growth through large-sized biopores implies that parts of the root are exposed to air, i.e., do not have contact to the surrounding soil, and are therefore not actively involved in water and nutrient acquisition. On the other hand, crop roots are frequently found in pores with such diameters (Athmann et al. 2013) and plant root penetration through compacted soil layers might be severely inhibited without biopores. Therefore, the presence of biopores can be regarded as favorable compared to the absence of biopores (Logsdon et al. 2013). Finally, it can be expected that there is an optimum density of large-sized biopores for both enhanced root growth and an improved shoot biomass development. This, however, can be presumed being highly dependent on soil moisture via changing the penetration resistance of the soil.

To investigate these questions, we conducted a pot experiment with spring wheat grown in columns each with a subsoil and a topsoil part, where various biopore densities and soil moistures were established. The following hypotheses were tested: (A) plants grown under comparatively moist subsoil conditions show an improved plant nutrient status as well as an improved hydration status which both positively affect shoot biomass. (B) Plants grown under a higher biopore density show an improved plant nutrient status as well as an elevated hydration status which both positively affect shoot biomass.

Material and methods

Experimental design

The experiment was performed using 60 columns (sewer base pipes) with an inner diameter of 18.9 cm and a height of 80.5 cm. The first 60 cm of the columns were filled with subsoil while the upper 20.5 cm were filled with topsoil. The bottom of the columns was covered with a perforated lid. The soil used for the experiment was an Albic Luvisol (according to the World Reference Base for Soil Resources) and was collected from the Teaching and Research Station of the Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences of Humboldt-University of Berlin (location coordinates: 52°28′07.5″N 13°17′43.5″E) with help of a mini excavator. The subsoil was collected in a depth range of 40–75 cm, and the topsoil was collected in a depth range of 0–25 cm. Soil chemical and physical properties are given in Table 1. Prior preparation of the columns, both the subsoil and the topsoil were dried, and the topsoil was sieved with a sieve mesh size of 0.5 cm and the subsoil was sieved with a sieve mesh size of 0.3 cm.

In a first step, the lowest part of the column was filled with a gravel-sand mixture of 10 cm height (Fig. 1).

Table 1 Soil physical and chemical properties of the topsoil and subsoil. Potassium, Mg, and P are given in mg 100 g^{-1} soil. Total carbon (C_t) and total nitrogen (N_t) are given in %

	Topsoil	Subsoil
Soil texture	Loamy sand[c]	Sandy loam[d]
pH	5.02	5.03
C_t	0.91	0.19
N_t	0.08	0.02
K_{DL}[a]	11.03	18.02
Mg_{CaCl2}[b]	5.07	8.21
P_{DL}[a]	23.13	4.20

[a]Examined by double-lactate extraction
[b]Examined by calcium chloride extraction
[c]83.60% sand, 14.69% silt, 1.71% clay
[d]69.43% sand, 16.52% silt, 14.06% clay

Afterwards, subsoil was filled in five steps resulting in 5 subsoil layers with a soil depth of 15–24 cm, 24–34 cm, 34–44 cm, 44–54 cm, and 54–64 cm (Fig. 1). In order to simulate environmental conditions with minimized water supply in the subsoil part of the columns, a subsoil moisture of 10% of the maximum water holding capacity (WHC) was set in 30 columns. This experimental group was referred to as WHC$_{10\%}$. In the other 30 columns, a subsoil moisture of 30% of the maximum WHC was set, referred to as WHC$_{30\%}$. For this, prior filling the soil into the columns, the WHC$_{100\%}$ of the sub- and the topsoil were determined by assessing the soil weight using calibrated cylinders of the fully water saturated soil (by water-saturation for 3 h with a subsequent draining of 2 h) and of the dry soil (by drying for 24 h at 105° C in a drying chamber). The WCH$_{100\%}$ was then calculated with the formula:

$$WHC_{100\%} = \frac{Weight\ saturated\ soil - Weight\ dry\ soil}{Weight\ dry\ soil} * 100$$

Fig. 1 Experimental setup. Factor A: moisture regime in the subsoil. Factor levels: 10% water holding capacity in the subsoil (WHC$_{10\%}$); 30% water holding capacity in the subsoil (WHC$_{30\%}$). Factor B: pore density in the subsoil: Factor levels 0, 1, 2, 3, and 4 pores column^{-1}. The columns consisted of a lower and an upper section separated by a layer of coarse gravel. The topsoil had a moisture regime of WHC$_{30\%}$ in all columns. Twelve plants of spring wheat were grown in each column for 61 days
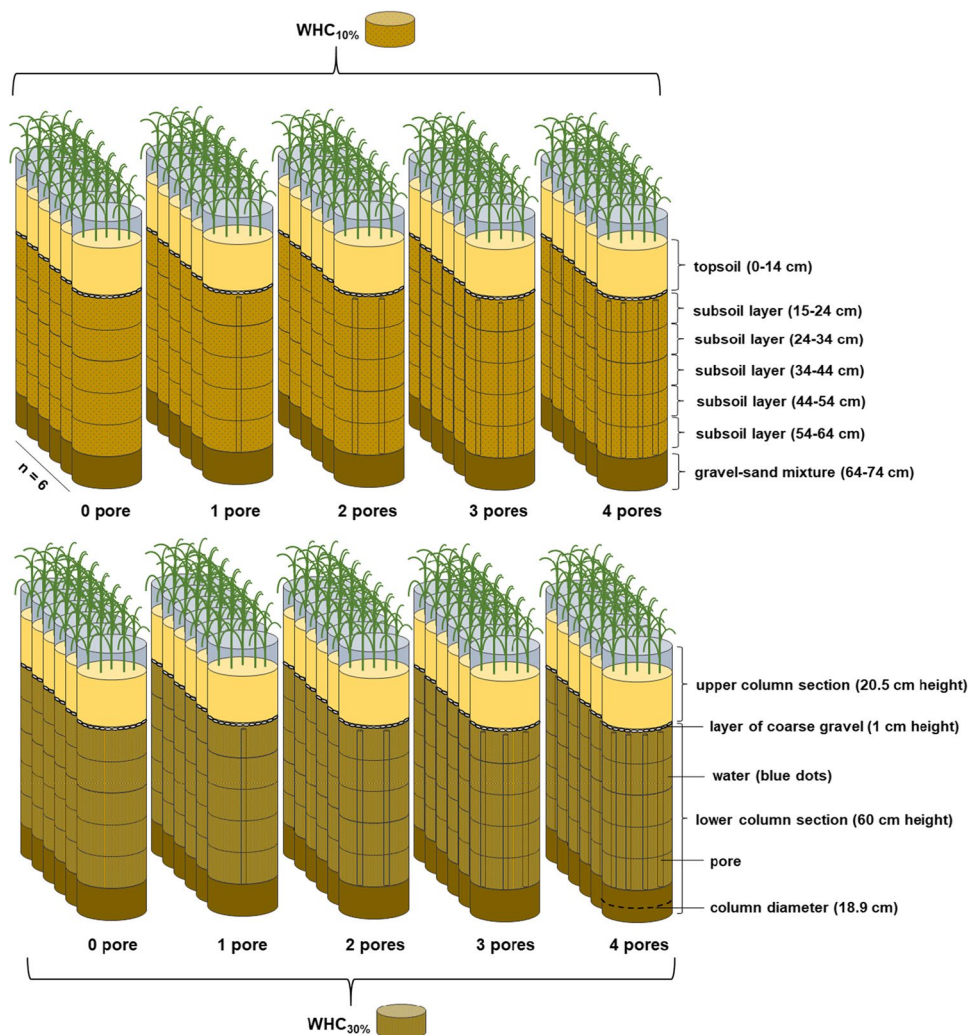
With help of this, the required water amount was calculated for setting a soil moisture of $WHC_{10\%}$ or $WHC_{30\%}$, respectively.

When preparing the columns, the subsoil was compacted to a density of 1.7 g cm^{-3} while the topsoil was compacted to a density of 1.4 g cm^{-3}. This was realized with help of a workshop press.

After filling the subsoil into the columns, artificial vertical pores with a diameter of 5.3 mm were created with help of an iron rod in the subsoil part. Previous studies have shown that this diameter is large enough to allow incubation with adult individuals of *L. terrestris* (Dresemann et al. 2018). Five different pore densities were set in each soil moisture regime: 0, 1, 2, 3, and 4 pores column^{-1} (Fig. 1). The pores were drilled in symmetrical distances to the center of the columns and the wall of the columns. The present experiment finally had 10 different treatments with 6 replications, resulting in 60 columns (Fig. 1). Next, one earthworm (*Lumbricus terrestris* L.; obtained from proinsects, Minden, Germany) was introduced into each pore. The subsoil and the pores were covered with a freshly harvested grass-clover mixture from the Teaching and Research Station of the Albrecht Daniel Thaer-Institute of Agricultural and Horticultural Sciences of Humboldt-University of Berlin. The purpose of introducing the earthworms into the pores was to line the pores with earthworm excrements in order to make the pore properties more natural for the subsequent plant growth. After 28 days, the earthworms were casted out of the columns by creating a heating gradient with help of a self-constructed heating chamber (ESM_1.pdf). A temperature of approximately 41 °C was reached in the lower parts of the columns, encouraging the earthworms to leave the columns upwards. The heating chamber was placed in a daylight-free environment as earthworms are very light-sensitive. Only a red lamp was used as light source in order to check if an earthworm left a pore. Each heating procedure lasted four hours. In order to treat all columns equally, also those columns without pores were heated for the same time. Soil water was lost by the heating procedure. Thus, the columns were weighed before and after the heating and water equal to the weight difference was added on the top of the subsoil part that seeped into the soil after a while.

After casting out the earthworms, the upper column part was fixed on top of the lower part with duct tape, and the subsoil layers were covered with a 1 cm thick coarse gravel layer in order to prevent the topsoil from trickling into the pores and to prevent capillary rise from the sub- to the topsoil. Finally, the topsoil was added in two steps on top of the coarse gravel layer with a final height of 14 cm. The soil moisture in the topsoil was set to $WHC_{30\%}$ in all 60 columns.

In order to document the soil moisture course, four time domain reflectometry (TDR) probes were installed in the subsoil part of one column of each treatment. The first probe was installed 14 cm above the column bottom. The further three probes were placed in a distance of 10 cm from the lower probe. The soil moisture was recorded three times per week from 18th of April until 27th of May 2019. The respective soil moisture values can be found in the supplementary file ESM_2.xlsx.

Finally, 12 plants per column of spring wheat (*Triticum aestivum* L. "Chamsin," KWS, Germany) were sown on 18th of April 2019. All 60 columns were randomly arranged and plants grew for 60 days in a covered but translucent outdoor installation. Mean temperature course, the relative humidity, and the sunshine duration during this growth period are given in ESM_3.pdf. After 30 days, all columns were completely randomized again. In the first 40 days of growth, all plants were watered via the topsoil equally according to demand. The soil-moisture in the subsoil remained unaffected by this (ESM_2.xlxx). During the last three weeks of plant growth, watering was stopped in order to simulate lacking precipitation. Holes were drilled into the bottom of each column wall and the columns were placed in shells (23 cm diameter and 7 cm high) which were filled with water to a height of 5 cm in order to simulate a water source in deeper soil layers. The gravel sand mixture in the lowest part of the columns prevented capillary water rise.

Due to a mildew (*Blumeria graminis*) infestation, a plant protection agent treatment with the agent Adexar® (active ingredient: Xemium; BASF, Ludwigshafen, Germany) took place on 15 May 2019.

Shoot and root sampling

On 17th of June 2019 (60 days after sowing), all shoots were cut at the base. At this time point, most of the plants had reached the end of ear emergence. The fresh weight of all shoot biomasses column^{-1} was recorded, and subsequently the plants were cut into small pieces. Each sample was homogenized, a subsample was taken and dried at 60 °C in a drying chamber for four days for later mineral analyses. The rest of the sample was dried at 105 °C in a drying chamber for four days in order to determine the dry substance and the dry matter yield.

From 18th until 25th of June 2019, sampling of the soil layers started. First, the upper column section was separated from the lower section and the topsoil was removed. Adhering gravel from the underlying gravel layer was removed, and each topsoil layer was packed in plastic bags and stored in a cooling chamber at 6 °C until the roots were washed out. Afterwards, the lowest gravel-sand layer was removed. Then, the five subsoil parts, starting from the lower part, were pressed out in steps of 10 or 9 cm, respectively, with help of the workshop press. Similar to the topsoil, each subsoil layer was packed into a plastic bag and subsequently stored in a cooling chamber at 6 °C until further processing.

Subsequently, the roots were washed out of the top- and subsoil layers using a sieve with a mesh size of 0.63 mm. The coarser roots were removed from the sieve using tweezers. The residue was re-suspended in water in a petri dish and the finer roots were separated with tweezers. The roots obtained in this way were placed in a plastic container filled with distilled water and stored in the refrigerator at 8 °C until root length scanning for a maximum of two days.

For the root scanning, the washed roots were evenly distributed in a plastic frame and fully covered with distilled water. The scanning was performed with a flat-bed scanner (EPSON Type 12000XL, EPSON, Meerbusch, Germany) in the photo mode. As document source "transparency attachment" and as template type "color positive film" were chosen. The image type was 8 bit grayscale with a resolution of 600 dpi and a high scan quality. The total root lengths in cm were received by evaluation of the scanned root images using the software WinRhizo Pro 64 bit. The total root length in cm was then converted into the root length density (RLD) per soil layer (cm cm^{-3}).

After the scanning, the roots of each soil layer were dried at 105 °C in a drying chamber for four days and the weight was taken to assess the dry matter yield of roots per soil layer.

For determination of the root/shoot ratio, the root weights of each soil layer were summed up per column, resulting in the root dry matter biomass per column. This was divided by the shoot dry matter yield (shoot + ear dry matter biomass).

Shoot mineral determination

Potassium (K), magnesium (Mg), and phosphorus (P) uptake in shoots were determined with inductively coupled plasma optical emission spectrometry (iCAP 6300 Duo MFC, Firma Thermo Fisher Scientific, Waltham, MA, USA). For this, 5 ml of 65% (v/v) nitric acid and 3 ml of 30% (v/v) hydrogen peroxide were added to 500 mg finely ground (with a vibrating mill: Schwingmühle MM 301, Retsch, Haan, Germany) and dried (at 60 °C) shoot material. The samples were digested in a microwave (Mars 6, CEM, Kamp-Lintfort, Germany) with the following program: 20 min at 200 °C and 2 min at 210 °C and 800 W and 30 min cooling. Finally, the samples were filled up to 50 ml with distilled water and filtrated to collect supernatant (Type 597, Whatman™, VWR International, Radnor, PA, USA). For the determination of total nitrogen (N), 300 mg of the same plant material as used for the determination of K, Mg, and P was weighed in crucibles and assessed by elementary analyses (Vario Max, Elementar, Hanau, Germany).

Shoot water content, transpiration, relative leaf water content, and proline concentrations

For describing shoot water relations, the total shoot water content, the transpiration per column, the relative leaf water content (RWC), and the proline concentrations in leaves were determined. The shoot water content was assessed immediately after harvest of the shoots and was defined as the weight difference of the fresh shoots and of the dried shoots after drying at 105 °C for four days.

The transpiration per column was determined at 59 days after sowing. For this, first the soil surface was covered carefully with cling film in order to exclude water loss via evaporation. Then, starting at 10 am, the weight of each single column was taken. After four hours, a second time the weights of all columns were taken in the same order as in the first measurement. The weight loss during this period was assumed to be the amount of water that the plant lost through transpiration and was calculated as mg water h^{-1}.

The RWC was likewise determined at 59 days after sowing and was performed as described by González and González-Vilar ([2001](#)) based on the original method by Barrs and Weatherley ([1962](#)). The RWC can be used as indicator for a water deficit in leaves and compares the initial and the turgid water content, on a percentage basis, of leaf disks punched from leaves.

The proline concentrations were determined as described by Woodrow et al. ([2017](#)). The fourth youngest leave of a single wheat plant per each column was harvested at 59 days after sowing. Subsequently, the leaves were freeze-dried in a freeze dryer (Christ Beta 1–16, Christ, Osterrode, Germany) for three days. Afterwards, the leaves were ground to a fine powder with help of a vibrating mill (Schwingmühle MM 400, Retsch, Haan, Germany). The proline determination itself was performed in the lab of the Department of Quality of Plant Products of the University of Hohenheim, Germany. First, leaf material was extracted with 70% (v/v) ethanol. The following methodology is based on the fact that proline reacts with the acidified dye ninhydrin and forms a blue-violet to red-brown color complex. The absorption of this complex can be quantified photometrically at a wavelength of 520 nm and with the help of a series of calibrators.

Statistical analysis

The statistical software R (Version 4.0.2, 2020) was used to evaluate the data using the following R packages: "gdata," "nlme," "piecewiseSEM," "multcomp," "lsmeans," and "car." The data evaluation started with the definition of an appropriate statistical model based on generalized least squares. The model included the factors "subsoil moisture," "pore number column^{-1}," and "soil layer" as well as all their interaction terms (two-way and

three-way). Hereby, the correlations of the measurement values due to the several levels of "soil layer," if applicable, were taken into account. The residuals were assumed to be normally distributed and to be heteroscedastic with respect to the different levels of "pore number column^{-1}" and "soil layer." These assumptions are based on a graphical residual analysis. Based on this model, a Pseudo R^2 was calculated and an analysis of variances (ANOVA) was conducted, followed by multiple contrast tests (e.g., see Hothorn et al. 2008; Scharschmidt and Vaas 2009) in order to compare the several levels of the influencing factors, respectively.

Results

Root length density and root biomass

A higher subsoil moisture (WHC$_{30\%}$ > WHC$_{10\%}$) significantly enhanced belowground biomass (Fig. 2). This is true for RLD and root biomass in the subsoil (15–64 cm soil-depth, considered as a whole) as well as in the whole column soil profile (topsoil and subsoil together, 0–64 cm). When additionally considering each soil layer separately, significant mean differences between the two subsoil moistures WHC$_{30\%}$ and WHC$_{10\%}$ for RLD and root biomass occurred in the subsoil layers 15–24 cm, 24–34 cm, 34–44 cm, and 44–54 cm soil-depth. Furthermore, significant mean differences occurred among the different soil layers of one
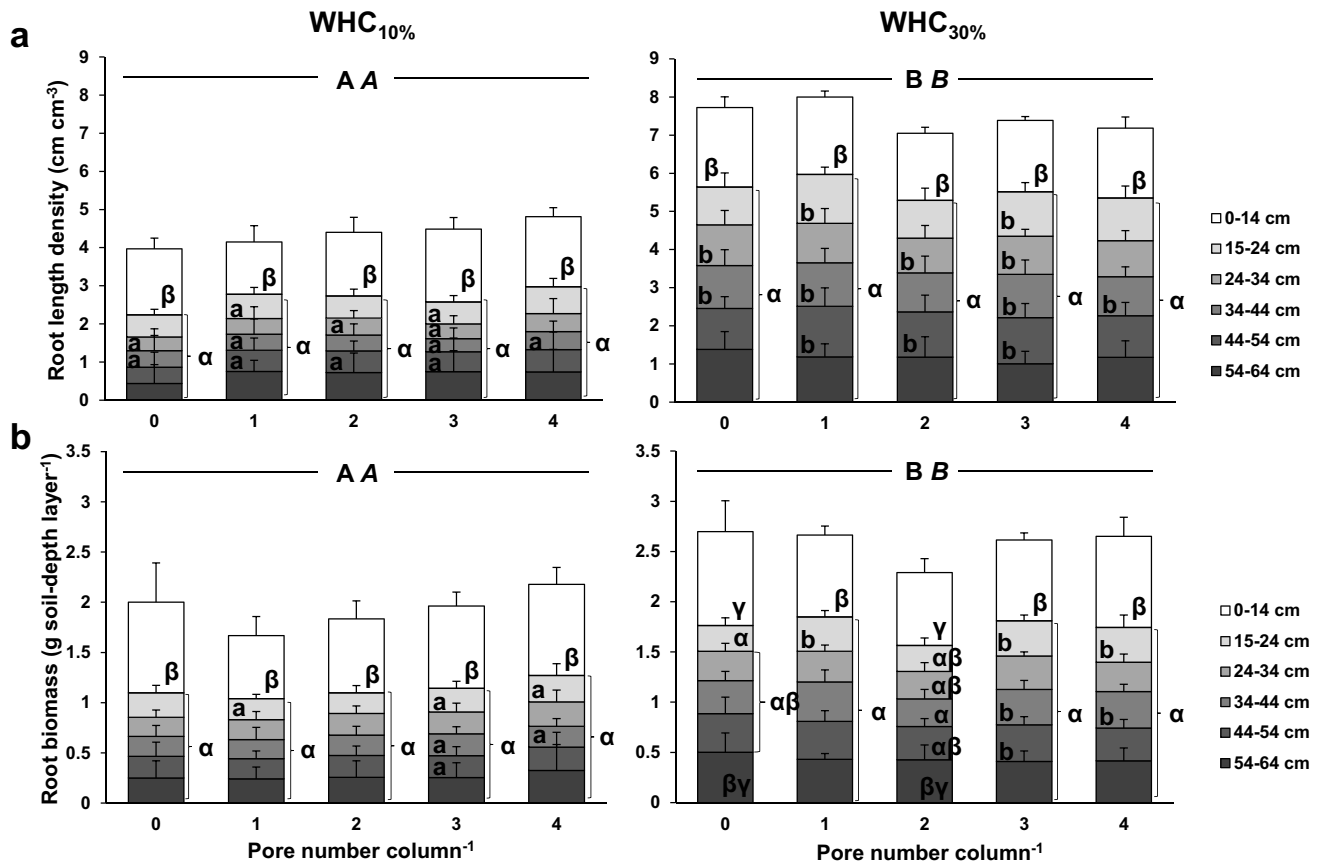

Fig. 2 Top- and subsoil root growth is differently affected under five different subsoil pore densities and two varying subsoil moistures. (a) Root length density (RLD) and (b) root biomass under a subsoil moisture of 10% water holding capacity (WHC$_{10\%}$) on the left and under a subsoil moisture of 30% water holding capacity (WHC$_{30\%}$) on the right side, under five different pore numbers column^{-1} (0, 1, 2, 3, and 4 pores column^{-1}), and in six varying soil depth layers (0–14 cm depth = topsoil layer; 15–24 cm, 24–34 cm, 34–44 cm, 44–54 cm, and 54–64 cm soil-depth = subsoil layers) at harvest (n = 6). Mean ± SE values. Capitals = significant mean differences of subsoil and topsoil (0–64 cm) between WHC$_{10\%}$ and WHC$_{30\%}$ averaged over all pore numbers column^{-1}. Italic capitals = significant mean differences of subsoil (15–64 cm soil-depth) between WHC$_{10\%}$ and WHC$_{30\%}$ averaged over all pore numbers column^{-1}. Small letters = significant mean differences between WHC$_{10\%}$ and WHC$_{30}$ of one pore number column^{-1} and one subsoil layer. Greek letters = significant mean differences between soil layers of one subsoil moisture level and one pore number column^{-1}. Level of significance for mean differences determined by multiple contrast tests is $p < 0.05$

treatment: The significantly highest RLD and root biomass was without exception located in the topsoil layer. Here, for instance, the topsoil root biomass considered as a proportion of the total root biomass of the columns was greater for columns with a subsoil moisture of $WHC_{10\%}$ with 41% compared to columns with a subsoil moisture of $WHC_{30\%}$ with 32%. Besides, the RLD as well as the root biomass were higher in the lowest subsoil layer (54–64 cm soil-depth) compared to other subsoil layers in columns with a subsoil moisture of $WHC_{30\%}$ (Fig. 2 a and b; right chart each).

Biopore density significantly impacted RLD and root biomass according to analysis of variance (ESM_4.pdf). However, the more specific multiple contrast test did not reveal significant differences between different pore densities. Still, in columns grown under a subsoil moisture of $WHC_{10\%}$, a clear tendency towards increasing RLD can be observed with increasing pore number column^{-1} (Fig. 2a; left chart). This increase can be reported for both subsoil RLD (15–64 cm soil-depth, considered as a whole) and total RLD of the column (topsoil and subsoil together, 0–64 cm). The root biomass of these plants did not show such a trend. Both, the increase in RLD with increasing number of pores column^{-1} and the missing effect on the root biomass was also reflected in the specific root lengths which was enhanced in columns with pores compared to columns without pores (ESM_5.pdf). No effect of the pore number column^{-1} could be documented in columns with a subsoil moisture of $WHC_{30\%}$, neither for RLD nor for root biomass (Fig. 2 a and b; right chart each).

Aboveground biomass

Similar to the belowground biomass, aboveground biomass was significantly enhanced when plants grew under a higher subsoil moisture. A higher subsoil moisture ($WHC_{30\%} > WHC_{10\%}$) led to on average 50–100% higher shoot as well as ear biomasses (Fig. 3 a and c). Besides, shoot biomass was significantly influenced by the subsoil pore number column^{-1}, however only in plants grown under a subsoil moisture of $WHC_{30\%}$. Here, plants grown in columns with 3 and 4 pores compared to plants grown in columns with 0 pores revealed a significantly higher shoot biomass (Fig. 3a; right chart). Figure 3b is illustrating the described findings by representative phenotypes which show the aboveground biomass of the treatments "0 pore, $WHC_{10\%}$", "4 pores, $WHC_{10\%}$", "0 pore, $WHC_{30\%}$", and "4 pores, $WHC_{30\%}$".

Root/shoot ratio

The above described results of the above- and belowground biomasses are also reflected by the associated root/shoot ratios (Table 2). Plants grown under a subsoil moisture of $WHC_{10\%}$ had significant higher (2- to threefold higher) root/shoot ratios when compared with plants grown under a subsoil moisture of $WHC_{30\%}$.

Shoot hydration status

The different subsoil moistures also led to significant differences in the shoot hydration: Plants grown under a subsoil moisture of $WHC_{30\%}$ showed significantly higher shoot water contents and transpiration rates compared to plants grown under a subsoil moisture of $WHC_{10\%}$ (Fig. 4 a and b). The RWC was only higher in plants grown under a subsoil moisture of $WHC_{30\%}$ compared to plants grown under a subsoil moisture of $WHC_{10\%}$ when grown with 0 pores column^{-1} (Fig. 4c). The shoot proline concentrations showed an opposite trend: Plants grown under a subsoil moisture of $WHC_{10\%}$ revealed significantly higher proline concentrations when compared with plants grown under a subsoil moisture of $WHC_{30\%}$ (Fig. 4d).

Furthermore, an increase in the number of pores column^{-1} improved the shoot water content of plants grown under a subsoil moisture of $WHC_{10\%}$ as well as of plants grown under a subsoil moisture of $WHC_{30\%}$ (Fig. 4a). For instance, when comparing plants with 0 pores with plants with 4 pores column^{-1} under a subsoil moisture of $WHC_{10\%}$, an increase in shoot water content of 50% on average (Fig. 4a, left chart) and a threefold increase of the transpiration rate on average (Fig. 4b, left chart) can be reported. Transpiration rate and RWC were only improved with higher pore numbers column^{-1} when compared to 0 pores column^{-1} in plants grown under a subsoil moisture of $WHC_{10\%}$ (Fig. 4 b and c, left chart each). The proline concentrations revealed again an opposite trend with decreasing concentrations in columns with more than zero pores column^{-1}. Columns with 4 pores column^{-1} exhibited a 50% lower proline concentrations on average compared to the experimental group without pores (Fig. 4d, left chart). This biopore-density related effect was only detectable in plants grown under a subsoil moisture of $WHC_{10\%}$.

Shoot mineral status

Growing plants under a higher subsoil moisture also overall improved plant nutrient uptake (Table 3). Especially K, Mg and N uptake showed on average a twofold increase with a higher subsoil moisture. In addition, K, Mg and N uptakes increased when grown under an increasing number of pores column^{-1}, especially when comparing no pores with 3 and 4 pores column^{-1}. However, this was observed only under a subsoil moisture of $WHC_{30\%}$. Here, K uptake revealed on average a 50% increase, and Mg and N uptake showed on average a twofold increase.
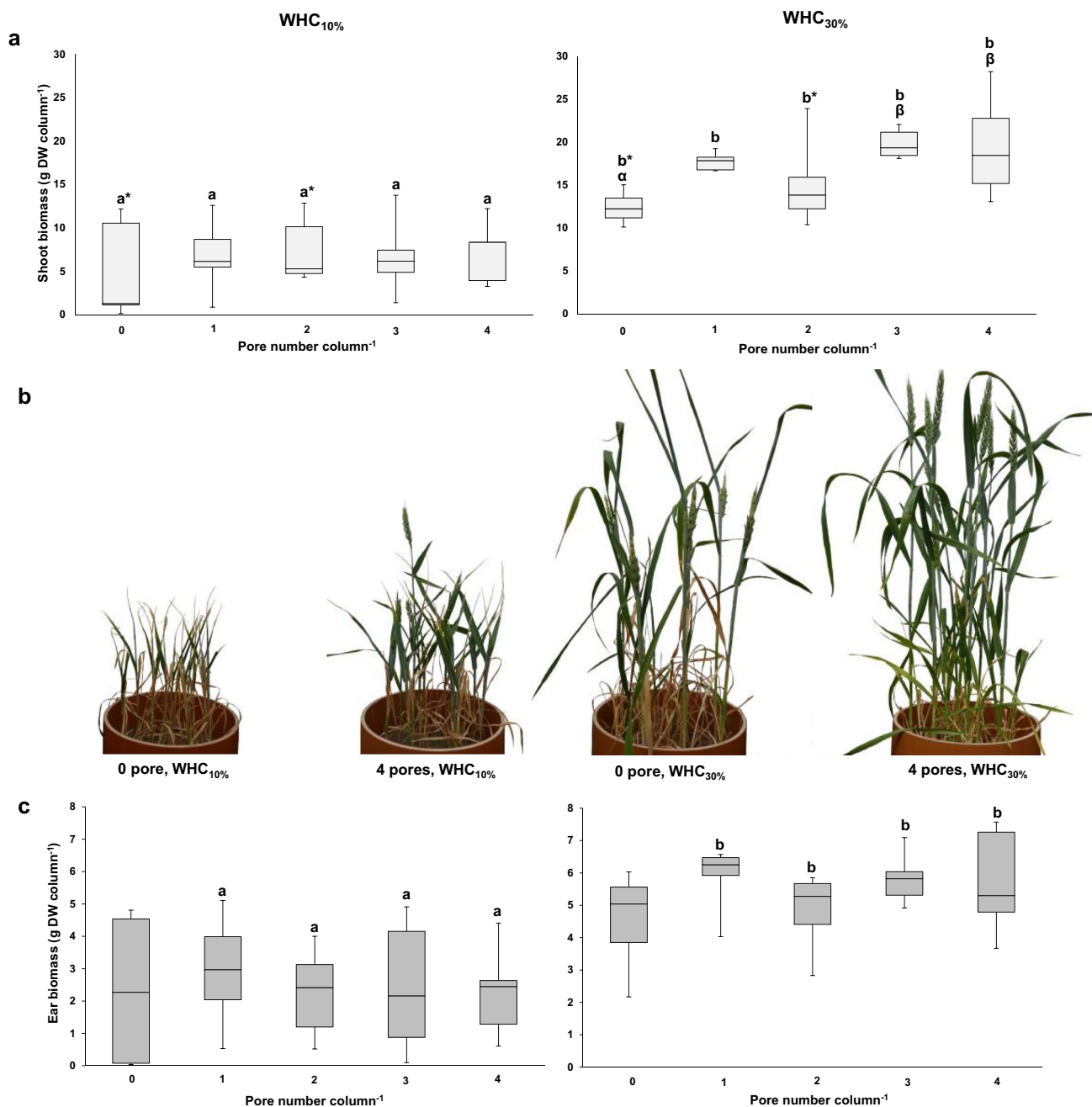
Fig. 3 The aboveground shoot biomass increases when grown under a higher subsoil moisture and an increasing subsoil pore number column^{-1}. (a) Shoot biomass under a subsoil moisture of 10% water holding capacity (WHC$_{10\%}$) on the left and under a subsoil moisture of 30% water holding capacity (WHC$_{30\%}$) on the right side and under five different pore numbers column^{-1} (0, 1, 2, 3, and 4 pores column^{-1}) at harvest ($n=4$–6). (b) Representative phenotypes showing the aboveground biomass of the treatments "0 pore, WHC$_{10\%}$", "4 pores, WHC$_{10\%}$", "0 pore, WHC$_{30\%}$", and "4 pores, WHC$_{30\%}$" 57 days after sowing. (c) Ear biomass under a subsoil moisture of 10% water holding capacity (WHC$_{10\%}$) on the left and under a subsoil moisture of 30% water holding capacity (WHC$_{30\%}$) on the right side and under five different pore numbers column^{-1} (0, 1, 2, 3, and 4 pores column^{-1}) at harvest ($n=4$–6). Mean $\pm$ SE values. Small letters = significant mean differences between WHC$_{10\%}$ and WHC$_{30\%}$ for one pore number column^{-1}. Greek letters = significant mean differences between 0, 1, 2, 3, or 4 pores column^{-1} for one subsoil moisture. Level of significance for mean differences determined by multiple contrast tests is $p<0.05$; * = $p<0.1$

Table 2 Root/shoot ratio under a subsoil moisture of 10% water holding capacity (WHC$_{10\%}$) and under a subsoil moisture of 30% water holding capacity (WHC$_{30\%}$) and under five different pore numbers column^{-1} (0, 1, 2, 3, and 4 pores column^{-1}) ($n=4$–6). Mean $\pm$ SE values. Capitals = significant mean differences between WHC$_{10\%}$ and WHC$_{30\%}$ averaged over all pore numbers column^{-1}. Level of significance for mean differences determined by multiple contrast tests is $p < 0.05$

| pore number column^{-1}	WHC$_{10\%}$		WHC$_{30\%}$	
0	0.79 ± 0.62		0.22 ± 0.06	
1	0.69 ± 1.08		0.15 ± 0.02	
2	0.28 ± 0.13	A	0.16 ± 0.05	B
3	0.37 ± 0.16		0.13 ± 0.01	
4	0.46 ± 0.30		0.13 ± 0.05	

Discussion

Effect of subsoil moisture

Growing plants in columns with a higher subsoil moisture significantly increased RLD and root biomass in the subsoil (Fig. 2). The increase in root growth in the subsoil might be ascribed to a decreasing penetration resistance in a moister compared to a dry soil (Volkmar 1996; Glinski 2018). Thus, roots have easier access to deeper soil layers and probably also to water sources located in the deeper subsoil. In the present study, roots had access to such a water source by placing the columns in shells filled with water. Supplementary Figure ESM_6.pdf shows that roots passed the lowest layer of gravel and thus had access to the provided water source. However, root biomass and RLD were not examined in the lowest gravel layer and in the shells filled with water. It is assumed, that (i) the improved access of the roots to deeper soil layers and finally (ii) to this water source, as well as (iii) a higher water supply via the higher subsoil moisture itself led to an improved hydration of plants grown under a subsoil moisture of WHC$_{30\%}$ compared to those grown under a subsoil moisture of WHC$_{10\%}$. This is reflected in significantly higher shoot water contents (Fig. 4a), higher transpiration rates (Fig. 4b) and higher relative leaf water contents (Fig. 4c) in plants grown under a subsoil moisture of WHC$_{30\%}$ compared to plants grown under a subsoil moisture of WHC$_{10\%}$. Plants grown under a subsoil moisture of WHC$_{30\%}$ also exhibited significantly lower shoot proline concentrations (Fig. 4d). An increased accumulation of proline in plants is an often reported phenomenon in consequence of a stress event, especially osmotic stress, since proline can take on the function of an osmolyte (Dar et al. 2016). It should be noted, however, that the data discussed are based on only one point in time in the present study.

Therefore, it can only be presumed that the data on plant hydration are representative for earlier times of plant growth.

An increasing soil moisture may also have helped to improve the plant's nutrient acquisition which is shown by the overall higher shoot nutrient uptake of plants grown under a subsoil moisture of WHC$_{30\%}$ compared to plants grown under a subsoil moisture of WHC$_{10\%}$. Potassium, Mg, P, as well as N are mainly taken up by plants via mass flow driven transpiration via the soil solution (Barber 1995; Marschner 2011; Plhak 2003). Thus, mass flow driven nutrient uptake depends on water uptake which was more available in plants grown under a subsoil moisture of WHC$_{30\%}$ than in plants grown under a subsoil moisture of WHC$_{10\%}$. Therefore, the superior supply of the plant with water might also have led to a superior plant nutrient status. Similar to our findings, Volkmar (1996) indicated a positive relationship between a higher soil moisture level and shoot N uptake, and Dresemann et al. (2018) between a higher soil moisture level and shoot N and P uptake.

Also, the increase in RLD and root biomass may have led to an overall higher water and nutrient uptake of the plant. A further reason of higher K and Mg shoot contents of plants grown under a subsoil moisture of WHC$_{30\%}$ might be that these plants had on average a ten percent higher proportion of root biomass located in the subsoil compared to plants grown under a subsoil moisture of WHC$_{10\%}$. Here, in the subsoil, K and Mg concentrations were markedly higher compared to the topsoil (Table 1). Thus, the higher proportion of roots in the subsoil of plants grown under a subsoil moisture of WHC$_{30\%}$ might have contributed to a more uptake of K and Mg from the subsoil. Finally, plants grown under a subsoil moisture of WHC$_{30\%}$ showed not only an increase of the RLD and the root biomass but also of the aboveground biomass (Fig. 3) which likely can be related to an improved plant hydration status (since cell turgor is
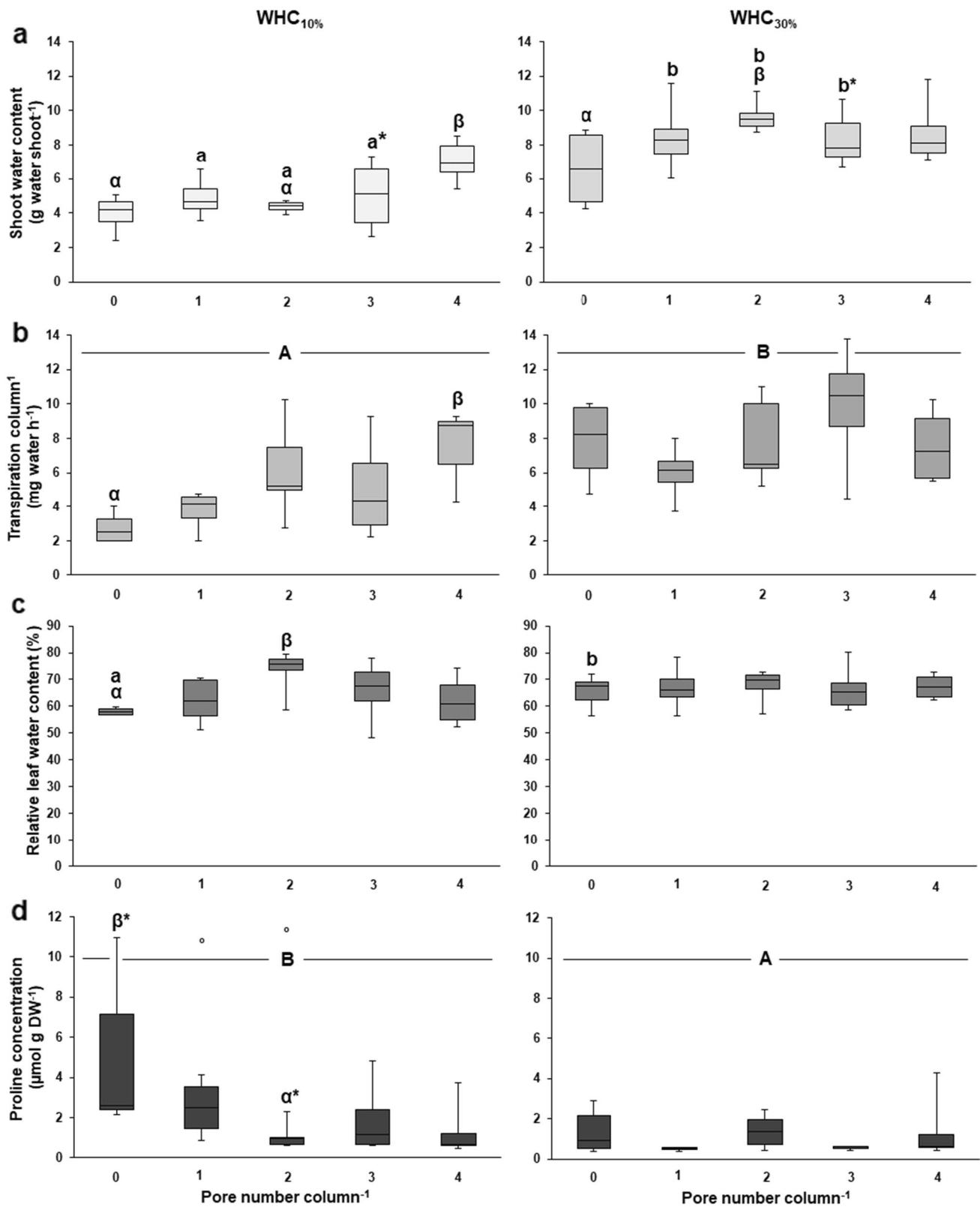
driving cell expansion as has been shown, for instance, by Cosgrove (2005)) as well as an improved nutrient status of these plants.

Plants grown under a subsoil moisture of $WHC_{30\%}$ revealed a 2- to threefold lower root/shoot ratio compared to plants grown under a subsoil moisture of $WHC_{10\%}$ (Table 2).

◄**Fig. 4** Indicators for the shoot hydration status are improved under a higher subsoil moisture and under an increasing subsoil pore number column^{-1}. (a) Shoot water content at harvest, (b) shoot transpiration column^{-1} 59 days after sowing, (c) relative leaf water content 59 days after sowing, and (d) proline concentrations 59 days after sowing under a subsoil moisture of 10% water holding capacity (WHC$_{10\%}$) on the left and under a subsoil moisture of 30% water holding capacity (WHC$_{30\%}$) on the right side and under five different pore numbers column^{-1} (0, 1, 2, 3, and 4 pores column^{-1}) (n = 4–6). Mean ± SE values. Capitals = significant mean differences between WHC$_{10\%}$ and WHC$_{30\%}$ averaged over all pore numbers column^{-1}. Small letters = significant mean differences between WHC$_{10\%}$ and WHC$_{30\%}$ for one pore number column^{-1}. Greek letters = significant mean differences between 0, 1, 2, 3, or 4 pores column^{-1} for one subsoil moisture. Level of significance for mean differences determined by multiple contrast tests is $p < 0.05$; * = $p < 0.1$. ° = outliers

This means, plants grown under a subsoil moisture of WHC$_{10\%}$ invested more in root rather than in shoot growth. The reason for a lower root/shoot ratio of plants grown under a subsoil moisture of WHC$_{30\%}$ might be attributable to, first, the higher water availability from the soil itself, and second, to a lower penetration resistance in columns with a subsoil moisture of WHC$_{30\%}$ compared to WHC$_{10\%}$, also resulting in improved water access. While the course of the TDR-measurements demonstrates a clear decrease in soil moisture in columns with a subsoil moisture of WHC$_{30\%}$ (ESM_2.xlsx, data sheet "WHC$_{30\%}$," columns with 0 pores) starting at the beginning of May, such a decrease in soil moisture is only barely visible in columns with a subsoil moisture of WHC$_{10\%}$ (ESM_2.xlsx, data sheet "WHC$_{10\%}$," columns with 0 pores). The decrease of the subsoil moisture in columns with a subsoil moisture of WHC$_{30\%}$ at the beginning of May presumably marks the point when roots grew from the topsoil to the subsoil. In columns with a subsoil moisture of WHC$_{10\%}$, this likely occurred much later and to a lesser extent. This is reflected, besides the lower decrease in subsoil moisture over the time, in a much higher proportion of root biomass located in the topsoil of plants grown under a subsoil moisture of WHC$_{10\%}$ (41% root biomass located in the topsoil) compared to plants grown under subsoil moisture of WHC$_{30\%}$ (32% root biomass located in the topsoil) (Fig. 2b). The reason for the delayed passing of roots from the topsoil to the subsoil in columns with a subsoil moisture of WHC$_{10\%}$ might be a higher water availability in the top—compared to the subsoil, and second, a lower penetration resistance for the roots in the topsoil. This, however, can only be presumed since we did not measure penetration resistance. In contrast, plant roots in columns with a subsoil moisture of WHC$_{30\%}$ likely reached earlier and with a larger share of roots the lowest subsoil layer and with it the provided water source. This is also shown by the proportionally higher RLD and root biomass in this lowest subsoil layer in columns with a higher subsoil moisture compared to columns with a lower subsoil moisture (Fig. 2 a and b).

Finally, the hypothesis that plants grown under comparatively moist subsoil conditions show an improved plant nutrient as well as hydration status which both positively affect shoot biomass can be confirmed.

Effect of pore density

An increasing number of pores column^{-1} did not reveal any clear effect on RLD or root biomass of plants grown under a subsoil moisture of WHC$_{30\%}$ (Fig. 2 a and b, right chart each). However, shoot biomass was significantly increased from zero to three and 4 pores column^{-1} (Fig. 3 a and b, right chart each). Opposite to this, plants grown in columns with a subsoil moisture of WHC$_{10\%}$ showed an increasing tendency in RLD but no clear effect on root biomass (Fig. 2 a and b, left chart each) and only a negligible effect on shoot biomass with increasing number of pores column^{-1} (Fig. 3 a and b, left chart each). Biopores have been shown to serve as preferential pathway of growth for plant roots in compacted soils (Chen et al. 2011; Ogilvie et al. 2021). The found data for RLD in the present study (Fig. 2a, left and right chart) indicate that an increasing number of pores column^{-1} facilitate an easier access of plant roots into the compacted lower subsoil layers especially under the dry subsoil conditions. The fact that root biomass did not increase while the RLD in the subsoil revealed a clear increase with increasing number of pores column^{-1} is also reflected in the specific root length (ESM_5.pdf). The increase in specific root length when comparing the columns having 1, 2, 3, or 4 pores column^{-1} with control plants (0 pore) (ESM_5.pdf, left chart), indicates that plants grown under the dryer subsoil conditions were able to explore a larger soil volume with the same amount of root biomass when there were pores available compared to no pores.

Due to the increased RLD, plant roots were likely able to use more of the available subsoil water. By facilitating the access of plant roots to the deeper soil layers with help of the pores, which probably also provided access to the water source below the columns, plant hydration status was improved. The improved plant hydration with a higher number of pores column^{-1} is shown by the higher shoot water contents, the higher transpiration rates and also a higher RWC when compared with no pores column^{-1} (Fig. 4a–c, left chart each). Moreover, the clearly lower proline concentrations of plants grown with an increasing number of pores column^{-1} when compared with the high proline concentrations of plants grown with no pores column^{-1} additionally shows that an increasing pore density has the potential to improve the plant's hydration status (Fig. 4d, left chart).

Nevertheless, the mean plant hydration of plants grown under a subsoil moisture of WHC$_{30\%}$ was overall superior compared to plants grown under a subsoil moisture of WHC$_{10\%}$ (Fig. 4). However, plants grown under a

Table 3 Potassium, Mg, P, and N shoot uptakes per column at harvest under a subsoil moisture of 10% water holding capacity (WHC$_{10\%}$) on the left and under a subsoil moisture of 30% water holding capacity (WHC$_{30\%}$) on the right side and under five different pore densities (0, 1, 2, 3, and 4 pores column^{-1}) ($n = 4$–6). Mean $\pm$ SE values. Capitals = significant mean differences between WHC$_{10\%}$ and WHC$_{30\%}$ averaged over all pore numbers column^{-1}. Small letters = significant mean differences between WHC$_{10\%}$ and WHC$_{30\%}$ for one pore number column^{-1}. Greek letters = significant mean differences between 0, 1, 2, 3, or 4 pores column^{-1} of one subsoil moisture. Level of significance for mean differences determined by multiple contrast tests is $p < 0.05$; $^*= p < 0.1$

WHC$_{10\%}$

pore number column^{-1}	Potassium	Magnesium	Phosphorus	Nitrogen
	g column^{-1} (in shoot DW)			
0	0.206 ± 0.249 ⌉	0.010 ± 0.011	0.019 ± 0.253	0.080 ± 0.078
1	0.242 ± 0.141	0.010 ± 0.057 a	0.016 ± 0.080 b	0.062 ± 0.037 a
2	0.277 ± 0.111 ⊢ A	0.011 ± 0.041	0.021 ± 0.037	0.128 ± 0.060
3	0.165 ± 0.109	0.007 ± 0.005 a	0.013 ± 0.009 a	0.059 ± 0.042 a
4	0.219 ± 0.111 ⌋	0.008 ± 0.004 a	0.015 ± 0.005 a	0.117 ± 0.046 a

WHC$_{30\%}$

pore number column^{-1}	Potassium	Magnesium	Phosphorus	Nitrogen
	g column^{-1} (in shoot DW)			
0	0.404 ± 0.060 α* ⌉	0.014 ± 0.004 α	0.037 ± 0.010	0.149 ± 0.044 α
1	0.583 ± 0.044	0.025 ± 0.004 b	0.042 ± 0.011 a	0.231 ± 0.338 b
2	0.487 ± 0.131 ⊢ B	0.021 ± 0.008	0.035 ± 0.013	0.222 ± 0.078
3	0.659 ± 0.084 β*	0.032 ± 0.007 bβ	0.042 ± 0.012 b	0.285 ± 0.061 bβ
4	0.688 ± 0.170 β* ⌋	0.031 ± 0.100 bβ	0.041 ± 0.013 b	0.311 ± 0.056 bβ

subsoil moisture of WHC$_{30\%}$ lacked the stimulating effect of biopores on RLD and on plant hydration. This demonstrates that a high biopore abundance may have the potential to improve RLD and plant hydration especially under dry subsoil conditions. This in turn may have the potential to increase also crop yield. In the present study there was no effect of an increasing biopore density on the aboveground biomass under comparatively dry subsoil conditions (WHC$_{10\%}$). However, in a microcosm study using soil columns with or without artificial continuous vertical macropores and varying soil moisture, presence of macropores generally had a positive effect on shoot dry matter and N uptake of wheat, which was especially pronounced under dry conditions (Dresemann et al. 2018). Beside, in a simulation approach based on data from field experiments, the impact of different taprooted and fibrous rooted precrops on spring wheat growth, water and nutrient uptake and grain yield under varying weather conditions was investigated (Seidel et al. 2019). In their study, spring wheat yield was clearly enhanced after lucerne, while among the non-leguminous precrops, high biopore densities after chicory were favorable in dry years but disadvantageous in years

with regular precipitation. Furthermore, in a field study by Kautz et al. (2015) barley revealed significantly higher grain yields following the biopore-forming chicory compared to the previous crop tall fescue during a year with very low precipitation.

Plants grown under a subsoil moisture of $WHC_{30\%}$ exhibited significantly higher K, Mg, and N uptake with an increasing number of pores column^{-1} (Table 3). This was, however, not the case for plants grown under a subsoil moisture of $WHC_{10\%}$. There are other studies showing that the drilosphere exhibited higher concentrations of N (Hoang et al. 2017), P (Bauke et al. 2017), K (Tiwari et al. 1989), organic C (Hoang et al. 2017), calcium, and some micronutrients (Pankhurst et al. 2002) if it was shaped by earthworms. This underlines the potential relevance of biopores for plant nutrition. Although the nutrient content of the drilosphere was not measured in the present study, it can be presumed that an increasing number of pores column^{-1}, which have been previously coated by earthworms, contributed to a better supply of K, Mg, and N also in this experiment (Table 3). Earthworms directly influence the nutrient content of the drilosphere mainly via digestion and excretion of their intestinal and cutaneous mucus, dependent on their food choice. However, there is increasing evidence that earthworms also indirectly influence the soil nutrient content via changing the soil microbial community (Medina-Sauza et al. 2019). Hereby, especially endogeic and anecic earthworm species are having stronger impact on soil nutrients compared to epigeic earthworm species (Medina-Sauza et al 2019). Hoang et al. (2016) could demonstrate that introduction of earthworms into by Chicory formed root biopores and earthworm formed biopores exhibited 1.2–3.9 times higher enzyme activities in the drilosphere which are involved in N and C cycle compared to just Chicory root-formed biopores without any earthworm influence. These enzymes are released by the earthworm-associated microbial community being involved in N and C turnover processes. Beside, these enzymes promote the degradation of complex soil organic molecules favoring the decomposition of soil organic matter which may promote nutrient release from the soil organic matter (Banfield et al. 2017; Bray et al. 2019). Likewise, P concentrations have been shown to have hotspots in the drilosphere which is also ascribed to an enhanced microbial activity which in turn is enhancing the solubility of P and thus the availability of P to the plant (Medina-Sauza et al. 2019; Vos et al. 2014).

Another effect which may impact on the nutrient and also water uptake of roots, may be the soil aeration. An impeded soil aeration significantly reduces root elongation which in turn negatively effects on nutrient and water uptake (Huang and Scott NeSmith 1999). Biopores can improve the soil aeration status (Ogilvie et al. 2021). Thus, the nutrient and water status of plants in the present study might have been improved in columns with increasing number of pores due to an improved soil aeration.

Finally, the second hypothesis that plants grown under a high pore density show an improved plant nutrient as well as hydration status which both positively influence on shoot biomass can only partly be confirmed. Plants grown under a subsoil moisture of $WHC_{30\%}$ indeed exhibited an improved nutrient status as well as shoot biomass with an increasing number of pores column^{-1}. However, the plant hydration was not improved by an increasing number of pores column^{-1} when grown under a subsoil moisture of $WHC_{30\%}$. Contrary, plants grown under a subsoil moisture of $WHC_{10\%}$ revealed an improved plant hydration with increasing number of pores column^{-1}, while there was no effect of biopores on shoot nutrient uptake and only a negligible effect on shoot growth.

Conclusions

The present study demonstrated that the presence of biopores can result in an improvement of the shoot hydration, the nutrient uptake and finally of the wheat aboveground biomass. This effect, however, strongly depended on the subsoil moisture. An improvement of the shoot hydration which can be related to an increased biopore density was only found in plants grown under the dry subsoil moisture regime. Under the dry subsoil moisture regime biopores led to an increase of the RLD enabling plants to access and take up more water in the deeper soil layers. This biopore-related effect was absent in columns with a moist subsoil regime, likely because here biopores did not represent pathways of lower resistance since we assume that the penetration resistance was overall lower in these columns due to the higher subsoil moisture. The improved shoot hydration due to an increased biopore density did not, however, lead to an improved aboveground biomass in this experiment. An improved nutrient uptake which can be related to an increased biopore density was only found in columns with a higher subsoil moisture. These plants also showed an increased shoot biomass which was likely caused by the increased nutrient uptake. The increased nutrient uptake in turn can be ascribed to direct and indirect effects of earthworms contributing to increase the nutrient content of the drilosphere. These nutrients could certainly be taken up better by the plant as result of mass flow which was more pronounced in the moister soil. Plants which grew under a lower subsoil moisture did not reveal a higher nutrient uptake with increasing biopore density, although there must have been the same effect of earthworms on nutrient content in the drilosphere. We guess that the fewer available water did not allow for mass flow-driven uptake of nutrients under lower subsoil moisture. Although an increasing biopore density revealed an improved shoot

hydration in plants grown under the lower subsoil moisture, it is likely that the increase in water uptake was not enough to increase also nutrient uptake. This should be validated in follow-up experiments. A further question which should be elucidated in follow-up studies is as to whether dynamic changes of the soil moisture due to continuous changes in precipitation influence on root and shoot growth in dependence on biopores.

Data availability Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

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