Root hairs matter at field scale for maize shoot growth and nutrient uptake, but root trait plasticity is primarily triggered by texture and drought

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Abstract

Aims Root hairs are important for uptake, especially for nutrients with low mobility in soils with high sorption capacity. Mutants with defective root hairs are expected to have lower nutrient uptake, unless they compensate with more root growth. Since root hairs can also contribute to the plant's water uptake their importance could change over the course of a growing season. It was our objective to investigate the role of root hairs under field conditions.

Methods The root hair mutant *rth3* of *Zea mays* and the corresponding wild-type were grown for two years under field conditions on sand and loam.

Results Shoot growth and P and K uptake of the plants were promoted by the presence of hairs at all growth stages. Differences between genotypes were greater on loam than on sand until tassel emergence, presumably as additional exploitation by hairs is more relevant in loam. Compensation for the absence of root hairs by increased root growth was not observed in absolute terms. The root to shoot ratio was higher for *rth3* than for wild-type. Root traits showed high plasticity in response to texture, the most salient

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being a greater mean root diameter in sand, irrespective of genotype. The mechanism causing the increase in mean root diameter is still unknown. Root length density was higher in sand, which can be explained by a greater need for exploration than exploitation in this substrate.

Conclusion The role of hairs for nutrient uptake could be confirmed under field conditions. The large impact of texture on root growth and consequences for carbon balance require further investigations.

Keywords Mechanical impedance · Nutrient availability · Rhizosphere · Root diameter · Root length density · Root to shoot ratio · Water availability · *Zea mays*

Abbreviations

AMF	Arbuscular mycorrhizal fungus
BBCH	BBCH-scale is a system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species
C	Carbon
CEC	Cation exchange capacity
DAP	Days after planting
K	Potassium
L	Loam
N	Nitrogen
P	Phosphorus
RD	Root diameter
RDW	Root dry weight
rth3	Root hair mutant
RV	Root volume
S	Sand
WT	Wild-type
WUE	Water use efficiency

Introduction

Root hairs

It has been demonstrated numerous times in controlled condition experiments that root hairs are important for nutrient uptake, in particular for those with low mobility like phosphorus (P) and potassium (K) (Bates and Lynch 2001; Bienert et al. 2021; Jungk 2001). A favourable role of hairs for water uptake, in particular under conditions of limited water availability, has also been suggested, but with contradicting results (Cai et al. 2021; Carminati et al. 2017a; Kwasniewski et al. 2016; Marin et al. 2020; Segal et al. 2008). Hairs increase the surface area for uptake and hence the soil volume influenced by an individual root at relatively low carbon costs (Gahoonia and Nielsen 1997; Lynch and Ho 2005). They have been shown to increase the size of the depletion

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zone for immobile nutrients, to facilitate diffusion of exudates like organic acids or exoenzymes, and, as a result, to alter microbiome composition (Gebauer et al. 2021; Marin et al. 2020 and citations therein). It is therefore expected that root hairs improve crop tolerance to abiotic stress such as low water and nutrient availability. As pointed out by Marin et al. (2020) most studies investigating the functions of root hairs have been conducted under controlled conditions, i.e. in sieved, homogenized soils, under artificial lighting, with limited soil volume, and for young growth stages only. Very few studies have attempted to validate these laboratory findings under field conditions (Gahoonia and Nielsen 2004; George et al. 2014; Marin et al. 2020; Nestler et al. 2016; Nestler and Wissuwa 2016; Ruiz et al. 2020). Among those, only the one referred to by Marin et al. (2020) and Ruiz et al. (2020) covered a range of plant parameters throughout the whole growing season including data on climatic conditions and soil water availability. In their trial root hairs did not confer a notable advantage to barley under optimal (nutrients and water) conditions. Yet, under soil water deficit root hairs improved plant water status and stress tolerance, while promoting shoot P accumulation. There is still a pressing need to conduct further field studies addressing the function of root hairs while continuously monitoring plant growth as well as water and nutrient status of plants and soils, including the effect of soil texture on such relations. However, such experiments need to take the interplay of different root traits into account, including possible compensation measures. They also have to disentangle in more detail which traits contribute to soil exploration and which ones will rather improve soil exploitation. Such studies are now possible, utilizing root hair mutants.

Plasticity of root traits

High plasticity in response to environmental conditions is a key property of roots and crucial for resource acquisition (Hodge 2004; Morris et al. 2017). The open questions include how a large number of traits are integrated upon environmental perturbation (Miguel et al. 2015; Wen et al. 2019; York et al. 2016), and when a particular trait is missing—which traits will be modified. For the

case of missing or short root hairs, some authors reported compensation by larger investment into root growth in general (Dodd and Diatloff 2016; Klamer et al. 2019) or fine roots in particular (Klamer et al. 2019). In a laboratory experiment comparing two maize genotypes (wild-type to the corresponding root hair mutant *rth3*) in two soil textures, a shift in root to shoot ratio could be confirmed but no plastic adaptation of root system architecture to the lack of root hairs (Lippold et al. 2021). However, both genotypes showed large plasticity of root architecture and in particular root diameter in response to texture. Whether these findings would hold under field conditions with unrestricted soil volume and fluctuating water supply is an open question.

Exploration and exploitation – the integration of root traits

Some root traits alter soil exploration, i.e. the size of the soil domain explored by roots, by modulating root architecture (i.e. axial root growth angle, growth rate, number of axial roots, lateral root branching) (Fitter 1987; Lynch 2013; York et al. 2013). At field scale, this can be measured as differences in the distribution of root length density over depth and its change over time. Another trait primarily related to exploration is the degree of mycorrhizal colonisation, supposed to reflect the abundance of extra-radical mycelium. Soil exploration can be distinguished from soil exploitation, i.e. how thoroughly resources are acquired within a given soil domain without further soil exploration (Fitter 1987). Root hairs improve soil exploitation similarly to other rhizosphere modifications, like the release of exudates, increased activity or number of mineral nutrient transporters, and enhanced root-soil contact (root diameter, mucilage, rhizosphere porosity) (York et al. 2013). The root length density within a given volume has an impact on exploitation as well. Exploitation strategies make a difference in environments with patchy, heterogeneous distribution of resources or a large proportion of nutrients with low mobility (Hodge 2006). In turn, exploration is expected to be more successful if resources are only available in low amounts throughout the soil profile or if resources are only available at larger depths (distance to the seed).

Hence, soil conditions like resource availability and distribution are expected to shape the plastic response to the lack of root hairs and the genotype specific integration of different root traits. For the latter, metabolic costs for different traits might be additional driving factors (Lynch et al. 2021). Experiments and *in silico* studies have shown that maintenance respiration comprises a significant part of plant carbon (C) budget; it can be lowered by aerenchyma formation, and it is also affected by root diameter and root hair formation (Jaramillo et al. 2013; Lynch and Ho 2005; Postma and Lynch 2011). At the field scale, apart from morphological and anatomical root traits, root to shoot ratio is a good indicator for plant C budget (Ordóñez et al. 2020).

Here, we present the results of a field experiment comparing a maize wild-type (WT) to a corresponding root hair mutant (rth3 – inhibited in root hair elongation) grown in two soil textures (loam, sand) to introduce variability in nutrient mobility. With this we expand a previous twin laboratory based experiment (Lippold et al. 2021) with the same treatments to the field scale following the challenge of upscaling laboratory based results (Vetterlein et al. 2021). As in the twin laboratory experiment nutrient supply was low, but the whole growing season under rain fed water supply was investigated instead of three weeks under optimal water supply. We tested the following hypotheses and expectations:

> H1: Hairs matter at field scale for biomass production and nutrient uptake, the effect is larger in loam where nutrient mobility is lower than in sand.
> H2: Root hairs contribute to plant water acquisition, in particular under drought stress. This results in a later onset of drought stress under water limiting conditions. As the mechanisms discussed are supposed to decrease water potential gradients in the rhizosphere, the effect will to be more relevant in sand compared to loam.
> H3: The root hair mutant will show plasticity in other root traits to compensate for the lower surface area. This is reflected in greater root to shoot ratio, increase in root length densities, larger share of fine roots and more intense mycorrhizal colonization. All of these will impact rhizodeposition

and, as a consequence, microbial composition and activity.

> H4: As initial differences in nutrient availability between textures are levelled by fertilisation, no specific texture related differences in root traits are expected.

Note that for some specific aspects related to the above listed hypotheses and expectations there are individual publications referring to the same field experiment and providing an in-depth discussion of the methods used and the results obtained. Here we will only refer to the results when appropriate. We refer to Jorda et al. for plant water relations, Ganther et al. for root gene expression, Santangeli et al. for root exudation and Rosskopf et al. for soil mechanical properties – all available in the same special issue.

Materials and methods

Experimental design

The soil plot experiment was carried out in 2019 and 2020 at the Bad Lauchstädt research station in Germany (51°23′36.10'' N; 11°52′30.29 E). Experimental design, maize genotypes, substrates, fertilization, agronomic measures and growth stages selected for sampling as well as monitoring devices for plant water relations are all described in detail in Vetterlein et al. (2021) along with the motivation of conducting twin experiments at laboratory and field scale in order to extrapolate results. Therefore, these topics are only briefly described here.

The basic design is a two factorial, randomised block design with six replications. Factor one is substrate (texture) with two levels (loam (L), sand (S)). Factor two is the *Zea mays* genotype with two levels comprising wildtype (WT), and a root hair mutant (*rth3*).

The monogenic mutant *rth3* is transposon induced and shows normal root hair initiation but disturbed hair elongation. The mutant shows no apparent aberrant shoot phenotype, but yield is reduced by 20 to 40% compared to the wildtype under field conditions (Hochholdinger et al. 2008). The mutated gene encodes a GPI-anchored COBRA-like cell wall protein RTH3 that is involved in the organization of the synthesized cellulose (Hochholdinger et al. 2018). The *rth3*

mutants used in these experiments are genetically highly homozygous because they have been back-crossed to the inbred line B73 for more than eight generations.

The substrate loam was obtained by excavating 700 t of a haplic Phaeozem soil (from 0 to 50 cm depth) in Schladebach, Germany (51°18′31.41″ N; 12°6′16.31″ E). The substrate sand was obtained by repeated mixing and sieving of the loam with quartz sand (550 t, WF 33, Quarzwerke Weferlingen, Germany). Setting up the field plots started right after the sieving operation in October 2018. Individual field plots (11 × 3.1 m) were excavated to a depth of 1 m. Vertical side walls were covered with a root barrier. The bottom of the plots was filled with a 25 cm gravel layer (0/32) and a drainage textile was then placed on top of the gravel. Substrates were then filled up to the original soil surface with a thickness of 75 cm. The loam was gradually placed in layers of 15 cm by a wheel loader, evened out with wheel loader bucket and compacted with a vibrating plate. This procedure proved to be suitable in a pilot experiment, as X-ray CT scans of extracted undisturbed soil cylinders showed no layering (Fig. 3 in (Vetterlein et al. 2021)). For treatment (S), sand was packed similarly; however, no vibrating plate was used.

The aim of fertilisation was to achieve a nutrient level in the range between slightly nutrient deficient to adequate nutrition for the wild-type genotype, in order for any investment into resource acquisition to pay-off. Initial differences in nutrient availability between substrates (Tab. S1) should ideally be compensated by fertilisation. As a result of pre-trials N, P, K, and Mg were added at a dose twice as high in sand compared to loam, and micronutrients were only applied to sand (Tab. S2). Fertilisers were surface applied, 50% prior to seeding, and the remaining 50% after first sampling. Fertilisation was the same in 2019 and 2020. Maize was sown to a depth of 5 cm. Distance within row was 20 cm; between rows 45 cm. This resulted in six rows with 54 plants each and a planting density of 9.5 plants m^{-2}; corresponding to a soil volume per plant of 78.935 dm^3 down to a depth of 75 cm. No pesticides were applied and weeding was done by hand. No heavy machinery was allowed to pass over the plots to avoid modification of soil structure. Soil cultivation in 2020 was only done by hand-hoeing

during weed control. Temperature and precipitation for the research station Bad Lauchstädt for 2019 and 2020 are provided in Fig. S1. Note that irrigation was required to allow germination in 2019 and to avoid damaging crop losses in 2019 and 2020.

For shoot and root sampling events specific growth stages according to BBCH-scale were selected (Bleiholder et al. 2001). BBCH-scale is a system for a uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species. The BBCH 14 growth stage (four leaves unfolded) was selected as the first sampling point as this corresponds to the developmental stage achieved after 21 days in the twin laboratory experiment (Lippold et al. 2021) – in 2019 BBCH 14 was reached 42 days after planting (DAP). The BBCH 19 growth stage (nine or more leaves unfolded) was selected as second time point representing exponential growth (DAP 63 in 2019). The BBCH59 growth stage (end of tassel emergence) was selected as time point during the transition from vegetative to generative growth (DAP 98 in 2019) and the BBCH 83 growth stage (early dough) as a growth stage representing ripening phase (DAP154 in 2019).

Soil water status was monitored throughout 2019 and 2020 with soil water content (TEROS 10; Meter Group AG) and soil water potential (TEROS 21 and TEROS 31; Meter Group AG) sensors installed in four depths (10, 20, 40 and 60 cm) in one representative plot per treatments (Vetterlein et al. 2021) (Jorda et al. submitted).

Shoot biomass sampling

At BBCH 14, 19 and 59 three representative plants per plot were sampled. The youngest unfolded leaves were separated and tissue was dried at 65 °C until constant weight. Youngest unfolded leaves and the remainder of the shoot were analysed separately. Nutrient concentrations in youngest unfolded leaves were used to derive plant nutrient status, analyses of remainder shoot and youngest leaves were used to calculate shoot nutrient content. Nutrient uptake was defined as shoot nutrient content divided by root surface area. For nutrient analyses tissue was chopped (Retsch SM2000) and subsamples were milled (30–50 min at 28/s with a

Retsch mill MM400). C and N were analysed by combustion with a CNS analyser (vario EL cube, Elementar, Germany). Approx. 50 mg plant material was weighed and used for determining P, K and Ca concentrations using an inductively coupled plasma optical emission spectrometry (ICP-OES, iCAP 6000, Thermo Fisher Scientific, Dreieich, Germany) after pressure digestion with nitric acid and hydrogen peroxide (ultraCLAVE V, MLS, Germany).

Root sampling

Three root cores per plot were taken at several depths between rows in 10 cm distance from the sampled plant foot. The motorized root corer (Humax Bohrsonden, Martin Burch AG, Switzerland) was equipped with a 20 cm long and 5 cm in diameter cartridge and samples were taken at 0–20, 20–40 and 40–60 cm depth, resulting in nine samples per plot which were washed over 0.65 mm sieves. Hereby, the sample was cleared of small stones and litter and the obtained roots were stored in a 50% alcohol solution (i.e. diluted Rotisol®). Subsequently, roots were scanned at 720 dpi with a 35 μm resolution using a flatbed scanner (EPSON perfection V700). Root traits were analysed using the software WinRhizo 2019 (Regent Instruments, Canada).

Sampling the remaining depth down to the drainage layer at 75 cm was omitted in order to avoid damage of the drainage fleece. For BBCH 14 only 0–20 cm depth was sampled. For BBCH 19 all depths were sampled in 2020, but in 2019 40–60 cm depth was not yet sampled.

In 2020 dead roots from 2019 were detected in sand treatments. Sorting out the dead roots by colour and appearance would have been too tedious and subjective. Hence, root degradation rate was estimated by a modelling approach assuming that most of the roots detected at BBCH 14 in 2020 were dead roots. In addition, it was assumed that degradation rate is temperature dependent and follows a first order kinetic. The model is described in detail in Jorda et al. submitted. Root length densities without correction are shown in Fig. S2.

Mycorrhization

After scanning with WinRhizo, the degree of mycorrhizal colonization was determined for subsamples consisting of ten fine root ($\varnothing < 1$ mm) segments. Fine roots selected were stained with ink (4001 Pelikan) after clearing roots in KOH (10%) (Vierheilig et al. 1998). For each sample, 100 fields of view were evaluated under light microscope. Following McGonigle et al. (1990), the presence of arbuscules, hyphae and vesicles was scored separately. Since the extent of hyphal colonisation correlated highly with the level of arbuscule formation ($p < 0.001$ according to ANOVA), and vesicle formation was rare in maize roots ($< 8\%$ of the roots), only the percentage of arbuscule formation is presented.

Rating of root hairs

The presence of root hairs was scored for the roots used to determine mycorrhizal colonisation, in order to confirm the presence and sum up the numbers of elongated hairs in wild-type and to assess if the *rth3* root hair elongation defect (Hochholdinger et al. 2008) was consistently expressed under field conditions.

Ratio of root cortex: vascular bundle

Root axes were randomly selected from the field samples used for WinRhizo scanning. Ten segments per plot were analysed for root diameter, for each segment ten free-hand cross sections were cut and analysed by light microscopy for root diameter and the diameter of the vascular bundle. The ratio of cortex to vascular bundle diameter was then calculated. For the cross sections evaluated the share of aerenchyma was scored. The scale used is provided with the respective figure.

Root to shoot ratio

The ratio of root fresh weight to dry weight and the ratio of root volume to fresh weight were determined by Oburger for the same field experiment (Oburger et al. personal communication).

From these ratios a conversion factor for root volume (RV) (determined with WinRhizo) to root dry weight (RDW) was derived: $RDW = RV*0.117$ g cm^3. Root dry weights from different depths were multiplied by the respective volume corresponding to an individual plant (derived from planting density) to calculate the root dry weight per plant. Shoot dry weight per plant was measured directly (see shoot biomass sampling).

Water use efficiency (WUE)

WUE was calculated using mean values of shoot (and root) dry weight divided by cumulative water flux from the soil. The data on cumulative water flux from the soil were taken from Jorda et al. submitted. Briefly, cumulative water losses from soil were estimated from volumetric water content measurements. The cumulative water loss was calculated using a soil water balance from the soil water storage change, and the precipitation and irrigation. As data for cumulative water flux from the soil are only available for one replicate per treatment, no statistics could be provided for WUE.

Statistics

For all figures, standard errors and mean values of six replicates (plots) are provided. Technical replicates within plots are not considered for statistics. A log-transformation was used prior to statistical analyses when normal Q-Q plots and Shapiro test indicated that the normal distribution criterion was not met. The software R version 3.53 (R Core Team 2018) and the libraries lme4, car, multcomp, ggplot and emmeans were used. A two-factorial ANOVA for the fixed factors substrate, genotype and their interaction was conducted in conjunction with Tukey's HSD test. The fixed factor depth was additionally used for some root trait data. Significant differences ($p < 0.05$) between treatments are displayed with different letters in the figures.
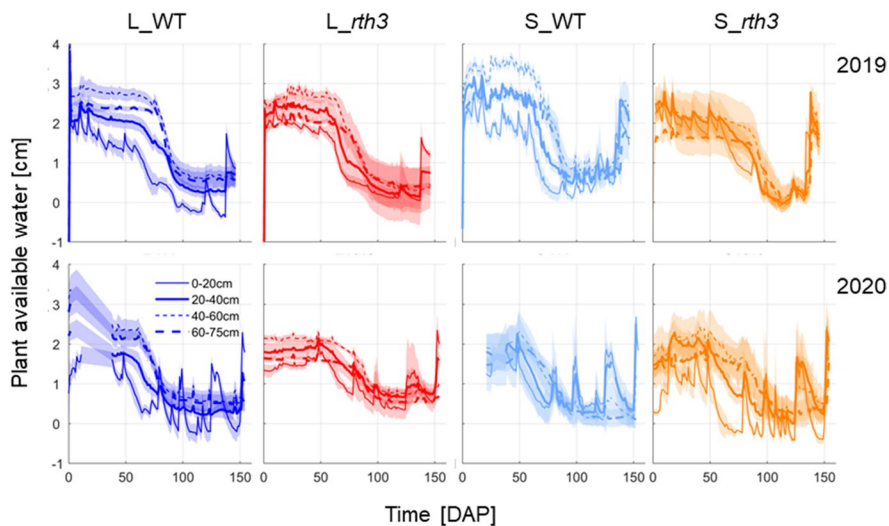

Fig. 1 Time course of plant available water during the growing season in 2019 and 2020 for loam and sand planted with wild-type and root hair mutant, respectively. Data are based on measurement in the respective soil depths. Note that growth stage BBCH 14 corresponds to 42 days after planting (DAP), BBCH 19 to DAP 63; BBCH 59 to DAP 98 and BBCH 83 to DAP154 in 2019. The semi-transparent band shows standard deviation, referring to n=3 sensors within the same depth in an individual plot

Results

Environmental conditions

Both years (2019, 2020) were characterized by low temperatures in May (BBCH 0—BBCH 14) and exceptionally high temperatures and low precipitation during June, July and beginning of August (Fig. S1). Weather conditions were slightly more extreme in 2019 compared to 2020. Soil matric potentials declined drastically between BBCH 19 (9 leave stage) and BBCH 59 (flowering), reducing plant available water to close to zero (pF 4.2) at flowering over the whole soil profile for all treatments, except S_rth3 (Fig. 1). In 2019, plants showed severe drought stress symptoms (leaf

rolling) and deviation of actual from potential transpiration in the following temporal sequence: L_WT (DAP 82) < L_rth3 (DAP 91) < S_WT (DAP 94) < S_rth3 (DAP 106) (Jorda et al. submitted). In 2020, deviation of actual from potential transpiration was observed slightly later in the season and in general earlier for the wild-type as compared to rth3 (L_WT (DAP 92) < S_WT (DAP 94) < L_rth3 (DAP114) < S_rth3 (DAP 138).

Shoot growth, shoot nutrient concentrations, content and nutrient uptake

During the early growth phase from germination to four leaves stage (BBCH 14) it was possible to compensate for the initial differences in nutrient

Fig. 2 Impact of substrate (loam, sand) and maize genotype (wild-type—WT, root hair mutant rth3–rth3) on plant nutrient status at different stages of plant development (BBCH 14, BBCH 19, BBCH 59) in the first year of soil plot experiment (2019) indicated by N, P, K concentration in youngest unfolded leaves. Statistics: two-factorial ANOVA in conjunction with Tukey's HSD test was conducted for each growth stage. Significant effect of factor is denoted by s for substrate, g for genotype and x for interaction. Differences between treatments (p < 0.05) are indicated by different lower case letters. Whiskers indicate standard error, n = 6. The grey shaded areas show the ranges for adequate supply according to (Bergmann 1986)
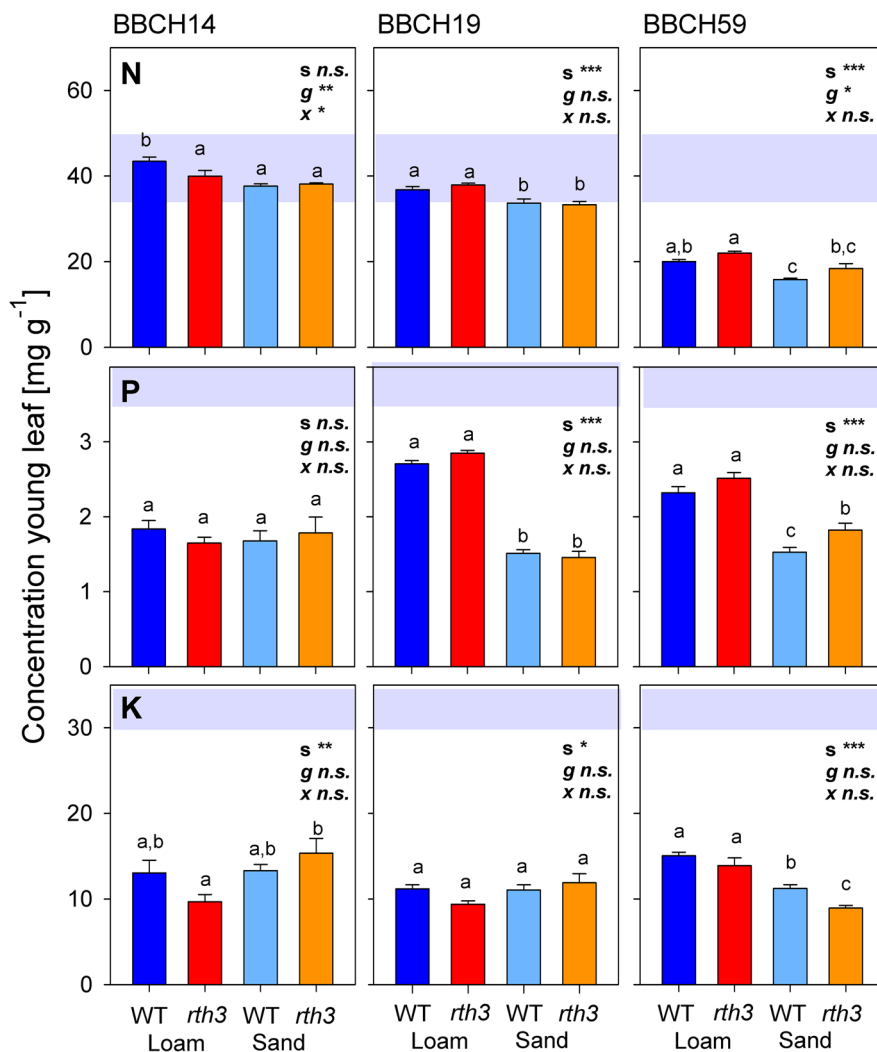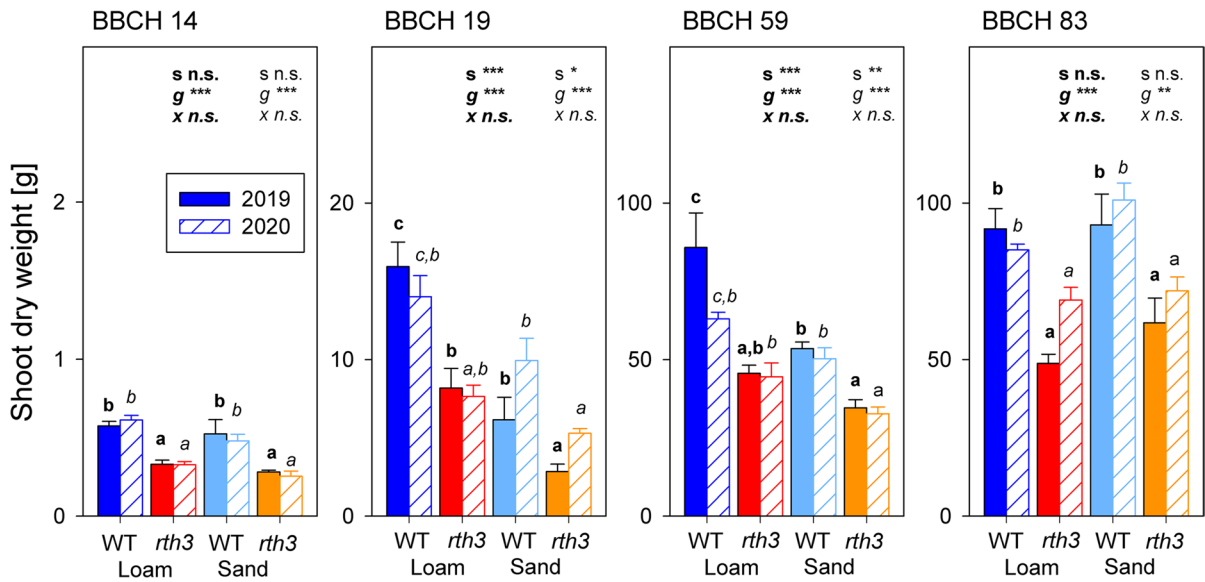
Fig. 3 Impact of substrate (loam, sand) and maize genotype (wild-type—WT, root hair mutant *rth3*–*rth3*) on shoot dry weight at different stages of plant development (BBCH 14, BBCH 19, BBCH 59, BBCH 83) in the first (2019) and second (2020) year of soil plot experiment. Note the different scales for the different growth stages. Statistics: two-factorial ANOVA in conjunction with Tukey's HSD test was conducted for each growth stage. Significant effect of factor is denoted by s for substrate, g for genotype and x for interaction. Differences between treatments (p < 0.05) are indicated by different lower case letters. Whiskers indicate standard error, n = 6. Bold letters refer to 2019, italic letters to 2020

supply through differential fertilisation of the two substrates. By this approach, the tissue concentration in the young unfolded leaves was not influenced (P, N) or only slightly influenced (K) by the substrate (Fig. 2). As intended, the plant nutrient status was below adequate supply, especially of P and K. During further plant development (BBCH 19, BBCH 59), however, nutrient deficiency increased more in the sand treatments compared to the loam treatments. For tissue concentrations, no significant influence of genotype was observed for any of the elements at any time during plant development, except for N at BBCH 14 and BBCH 59. In contrast to the tissue concentrations, the production of shoot biomass was significantly influenced by the genotype (Fig. 3). In both years and at all growth stages, the wild-type had a higher shoot dry weight than the mutant with defective root hairs. The substrate had a significant influence on shoot growth at BBCH 19 and 59, with the differences decreasing towards maturity (BBCH 83). In agreement with the dry weight of the shoots, the P content of the shoots (product of dry weight and concentration of shoot tissue) was significantly

influenced by the genotype at all growth stages (Fig. 4a). Especially in loam, the wild-type showed a higher P content than *rth3*, which was reflected in a significant influence of substrate and a significant interaction term at BBCH 19 and BBCH 59. P uptake per unit root surface area was also higher in the wild-type compared to *rth3* in loam, but not in sand (Fig. 4b). In general, the P uptake was higher in loam than in sand.

Root traits

Unlike shoot dry mass, root length density across all depths was significantly higher in sand than in loam at all growth stages except for BBCH 14 (Fig. 5). At BBCH 14 there was only a tendency for higher values for sand compared to loam; the values were generally low and the variability between the samples high. Genotype also had a significant effect on root length density: In the three depths, wild-type root length density was higher than that of *rth3* at BBCH 59 and BBCH 83 in sand. The differences between genotypes were smaller than those caused by substrate. In general, root length
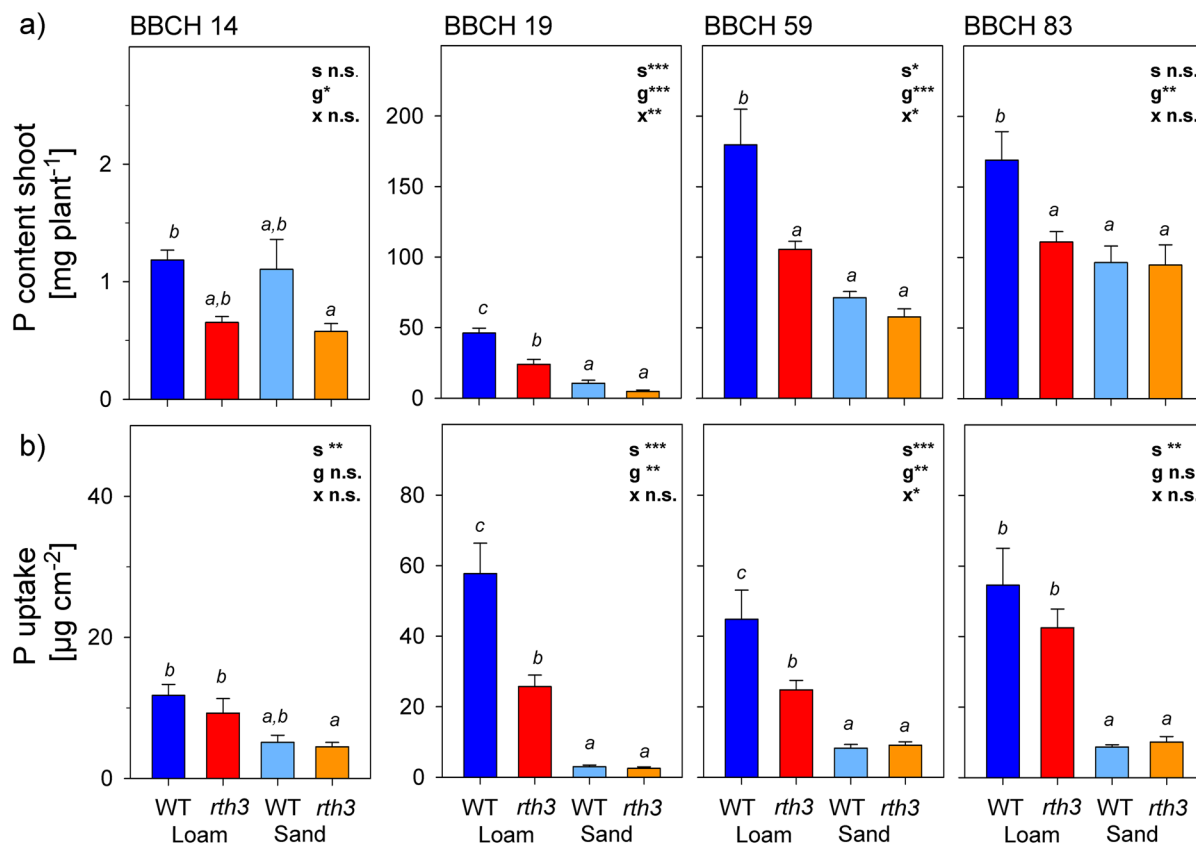
Fig. 4 Impact of substrate (loam, sand) and maize genotype (wild-type—WT, root hair mutant *rth3*–*rth3*) on shoot P content and P uptake per unit root surface at different stages of plant development (BBCH 14, BBCH 19, BBCH 59, BBCH 83) in the first year (2019) of soil plot experiment. Note the different scales for the different growth stages. Statistics: two-factorial ANOVA in conjunction with Tukey's HSD test was conducted for growth stage. Significant effect of factor is denoted by s for substrate, g for genotype and x for interaction. Differences between treatments ($p < 0.05$) are indicated by different lower case letters. Whiskers indicate standard error, n = 6

densities were higher in the top 20 cm in 2020 at BBCH 59 and 83. In 2020, it should be noted that the root length densities for sand were higher than for loam, despite the correction for dead roots. This correction was not necessary for loam as roots decomposed quicker in this substrate (see Fig. S2 for original values).

In 2019, the first year of plant growth on the newly established, homogenized plots, substrate had a significant impact on mean root diameter, with larger diameters observed for sand treatments as compared to loam treatments (Fig. 6). Genotype had a smaller impact on diameter, resulting in slightly larger values for *rth3* as compared to the wild-type. Significantly higher values for *rth3* than wild-type were observed e.g. in 0–20 cm depth at

BBCH19 and BBCH59 in sand during 2019, but in both substrates in 2020. Similar effects by genotype were observed at all depths.

In 2020, mean root diameters across all treatments were larger compared to 2019 (313 ± 17 μm versus 248 ± 3 μm). For 0–20 cm depth and partly for the lower depths the marked impact of substrate had vanished, whereas differences between genotypes became stronger in 0–20 and 20–40 cm depths.

Differences in mean root diameter between treatments were reflected in a shift in the ratio of cortex width in relation to the width of the vascular bundle (Fig. 7). Sand treatments had a higher ratio than loam in the 20–40 cm depth at BBCH 19 and 59 for both years. Interestingly, root segments
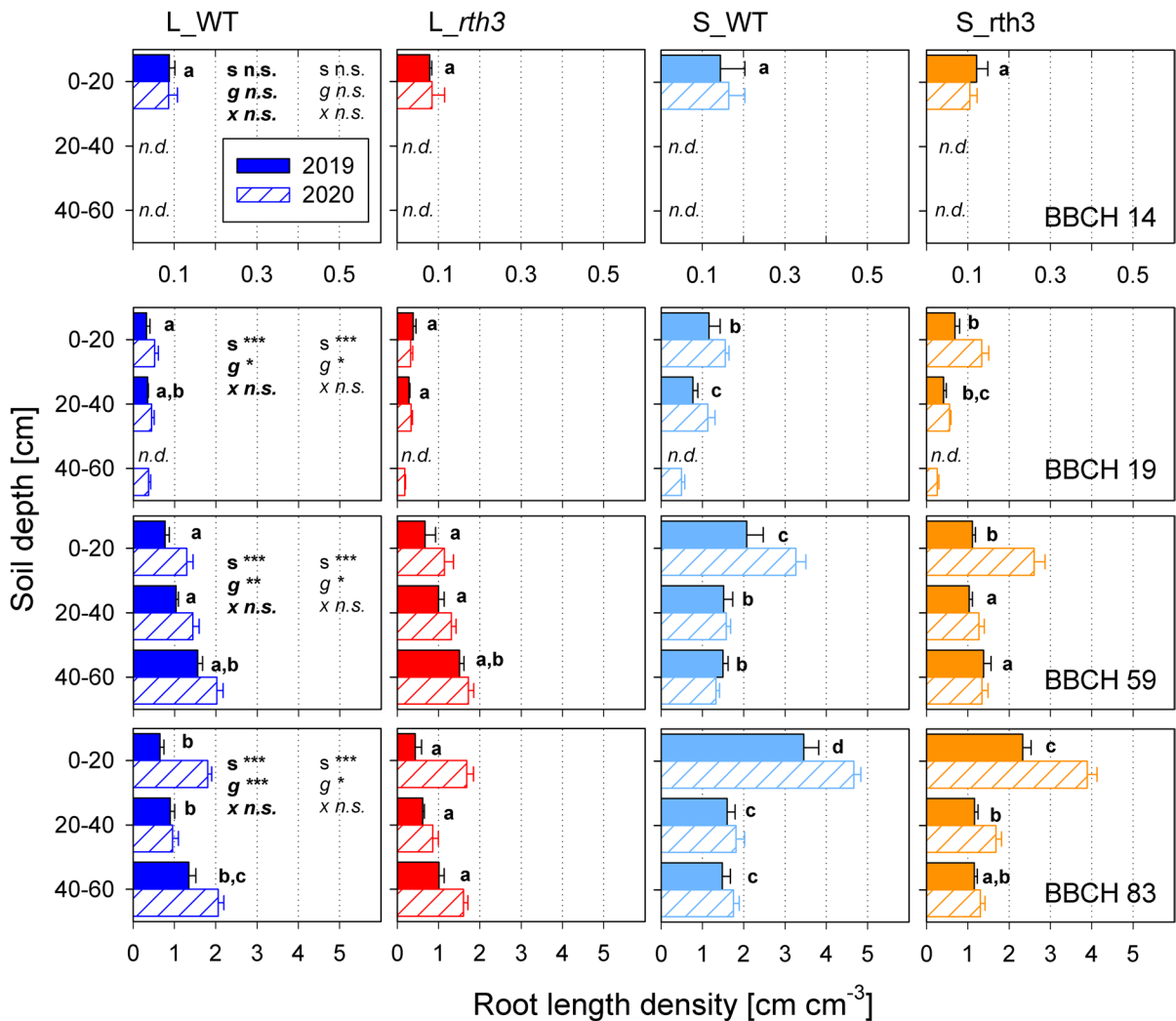
Fig. 5 Impact of substrate (loam, sand) and maize genotype (wild-type—WT, root hair mutant *rth3*–*rth3*) on root length density distribution with depth at different stages of plant development (BBCH 14, BBCH 19, BBCH 59, BBCH 83) in the first (2019) and second (2020) year of soil plot experiment. Note the different scales for the different growth stages; n.d. indicates that no values were determined. Statistics: two-facto-rial ANOVA in conjunction with Tukey's HSD test was conducted for growth stage, for each depth. Significant effect of factor across depth is denoted by s for substrate, g for genotype and x for interaction. Differences between treatments (p < 0.05) are indicated by different lower case letters. Whiskers indicate standard error, n = 6. Bold letters refer to 2019, italic letters to 2020

investigated likewise showed differences in aerenchyma formation, the latter being more marked for the loam treatment as compared to the sand treatments.

Mycorrhizal colonisation was observed as early as at BBCH 14 and it reached values in the range of 40 to 60% at BBCH 59 in 0–20 cm depth (Fig. 8). Colonisation rate increased by maize age and reduced by soil depth (p < 0.05). After no differences between substrates at BBCH 14, higher level of colonisation in loam than sand at BBCH 19 changed to a reversed pattern at BBCH 59 (Fig. 8). The impact of genotype was not significant.

The root hair mutant *rth3* consistently showed only few roots segments (< 20%) with some up to 50 μm extended hair like structures, but mostly only bulged epidermal cells, while 50–90% of
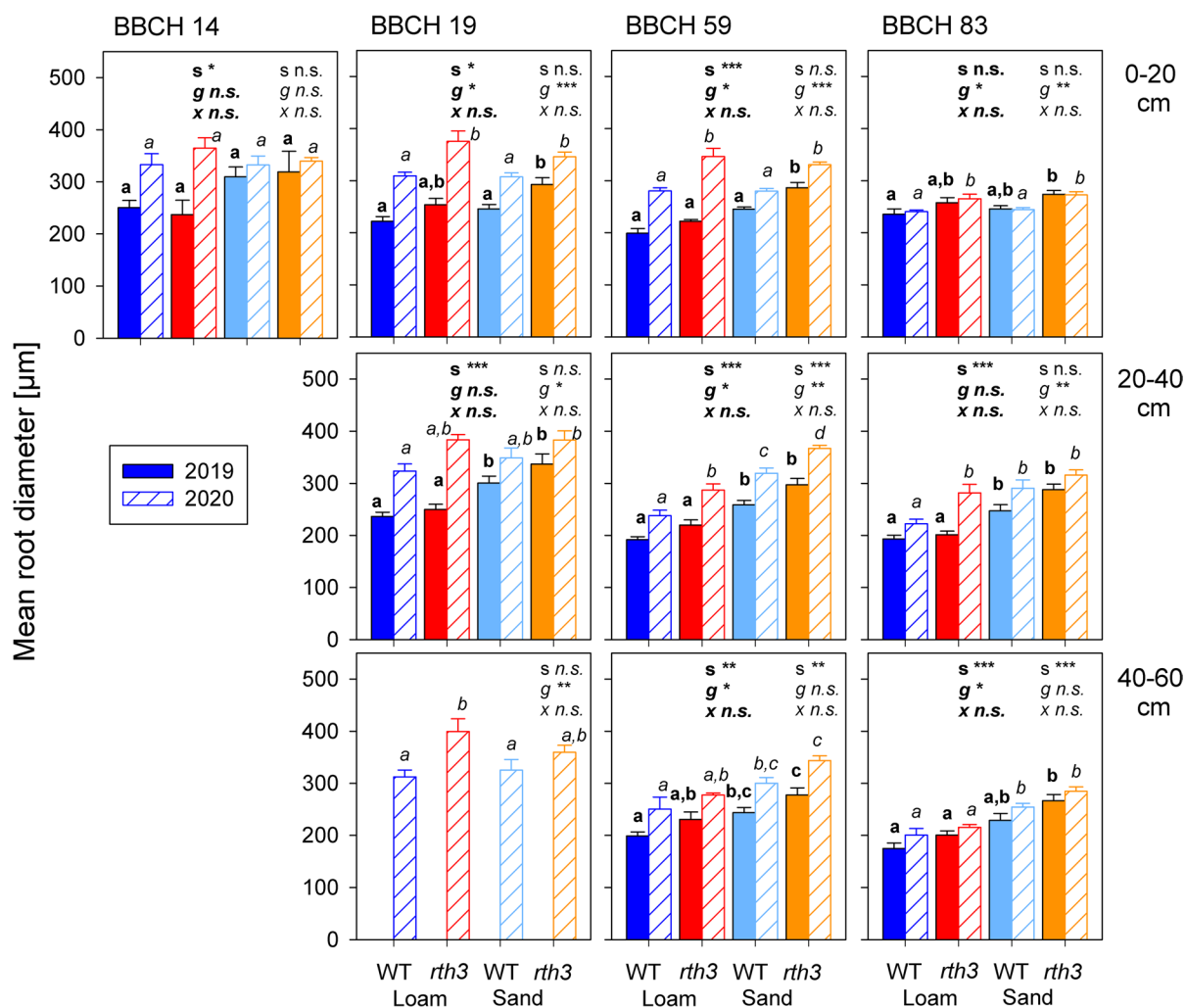
Fig. 6 Impact of substrate (loam, sand) and maize genotype (wild-type—WT, root hair mutant *rth3*–*rth3*) on mean root diameter in different depths, at different stages of plant development (BBCH 14, BBCH 19, BBCH 59, BBCH 83) in the first (2019) and second (2020) year of soil plot experiment. Statistics: two-factorial ANOVA in conjunction with Tukey's HSD test was conducted for growth stage, for each depth. Significant effect of factor is denoted by s for substrate, g for genotype and x for interaction. Differences between treatments for each depth (p < 0.05) are indicated by different lower case letters. Whiskers indicate standard error, n = 6. Bold letters refer to 2019, italic letters to 2020

wild-type root segments showed dense populations of elongated root hairs (Fig. S3).

Root to shoot ratio and water use efficiency

The different relevance of the factors substrate and genotype for shoot and root growth is reflected in the root to shoot ratio (Fig. 9). Substrate as well as genotype showed a significant impact on root to shoot ratio, with higher values for sand than loam and higher values for the root hair mutant *rth3* than the wild-type. In

line with what is expected during maize ontogeny, root to shoot ratios decreased with plant age. On average values in 2020 were 30% higher compared to 2019.

Water use efficiency, expressing water loss from the soil profile (transpiration plus evaporation) in relation to biomass produced during the respective period, increased with time. This is because the share of unproductive evaporation in relation to total water consumption decreased (Fig. 10, Jorda et al. submitted). Water use efficiency was always higher for the wild-type as compared to the root hair mutant *rth3*. This was
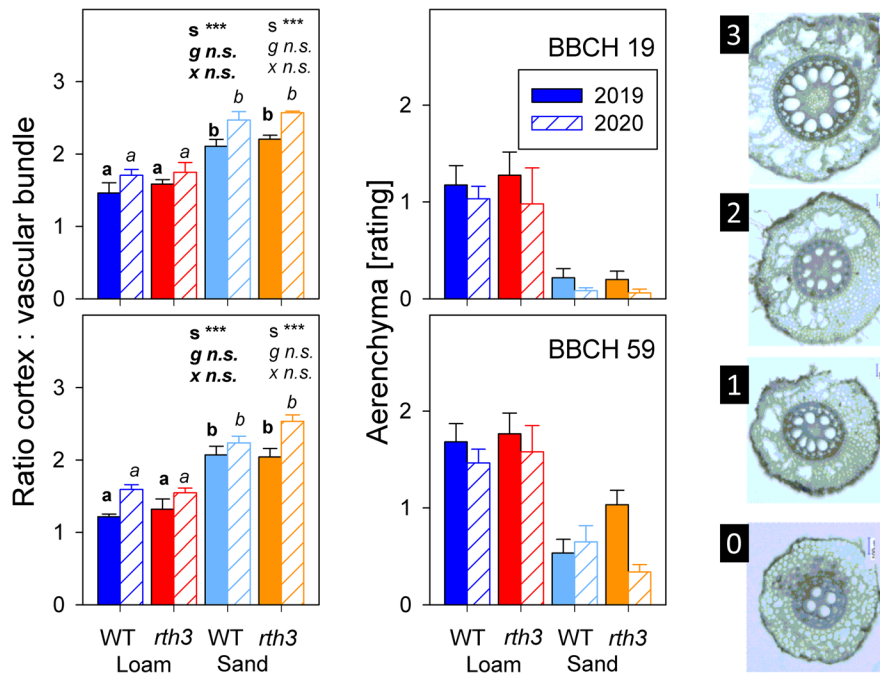
Fig. 7 Impact of substrate (loam, sand) and maize genotype (wild-type—WT, root hair mutant *rth3–rth3*) on the ratio of cortex width to width of the vascular bundle (left) and the degree of aerenchyma formation (centre) in 20–40 cm depth at two growth stages (BBCH 19, BBCH 59) in the first (2019) and second (2020) year of soil plot experiment. Scale used for aerenchyma scoring is indicated on the right. Statistics: two-factorial ANOVA in conjunction with Tukey's HSD test was conducted for growth stage. Significant effect of factor is denoted by s for substrate, g for genotype and x for interaction. Differences between treatments ($p < 0.05$) are indicated by different lower case letters. Whiskers indicate standard error, n = 6. Bold letters refer to 2019, italic letters to 2020

observed irrespective of substrate and year and is in line with observations in column experiments under well-watered conditions (Fig. 10, inset).

Discussion

For the discussion part of our study, we will attempt to answer our original hypotheses and expectations stated in the introduction. The overall pattern emerging is conceptualized in Fig. 11.

Do hairs matter at field scale for biomass production and nutrient uptake, in particular for loam as mobility of nutrients is low?

The relevance of root hairs for plant nutrient acquisition has been reviewed in depth by Jungk (2001) and was recently revisited by Bienert et al. (2021) providing an overview on the location of respective transporters for all essential plant nutrients. In line with literature, the observed differences in shoot biomass development between the wild-type and the root hair mutant corresponded to differences in shoot P content, and for the substrate loam that was accompanied by higher P uptake per unit root surface. Such higher normalized P uptake could reflect the expected upregulation of P transporters in the hairs under P deficiency (Bienert et al. 2021). However, they could also simply be explained by increased surface area for uptake provided by the hairs, or the decrease in path length required for P diffusion across the rhizosphere (Leitner et al. 2010). The latter is of particular relevance for soils with low P mobility and would explain why differences in uptake rates were not observed in sand, not even for the early growth stages. In sand neither P uptake nor P content differed between genotypes. The differences in shoot biomass are potentially explained by the higher investment of the root hair

Fig. 8 Impact of substrate (loam, sand) and maize genotype (wild-type—WT, root hair mutant *rth3–rth3*) on the colonisation of roots by arbuscular mycorrhiza for different depths at different growth stages (BBCH 14, BBCH 19, BBCH 59) in the first year (2019) of soil plot experiment. Statistics: two-factorial ANOVA in conjunction with Tukey's HSD test was conducted for growth stage and depths. Significant effect of factor is denoted by s for substrate, g for genotype and x for interaction. Differences between treatments (p < 0.05) are indicated by different lower case letters; n = 6
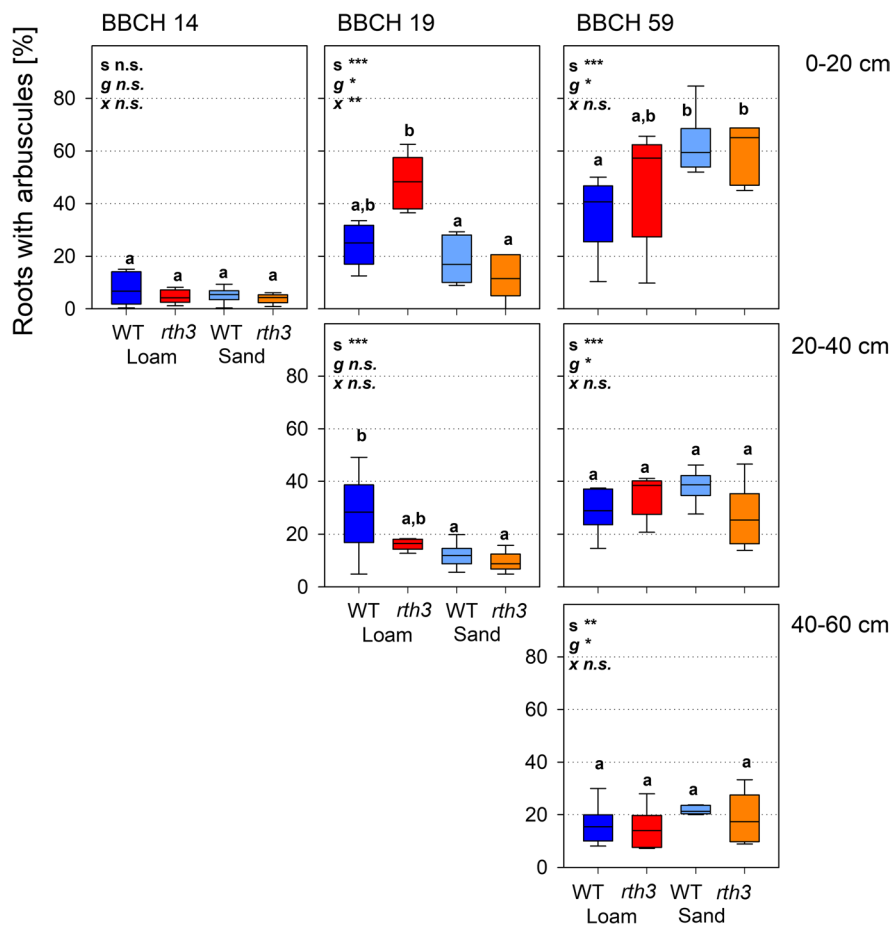
mutant into root growth (reflected in root to shoot ratio), which might have enabled similar P uptake. The P concentration in young leaves indicate that P availability was lower for sand during the later growth stages (BBCH 19, BBCH 59). For sand, unlike loam, initial plant available P concentration and total P concentration (Bilyera et al. 2021; Vetterlein et al. 2021) have been very low (8.3 and 53 mg kg^{-1} respectively). Thus, plants in the sand plots relied to a larger extent on surface-applied fertiliser, and when this was depleted or no longer available due to drying of the topsoil, the only viable adaptation strategy was to tap into a larger soil volume (Fig. 11). In loam, initial plant available P concentration was four times higher than in sand (32.7 mg kg^{-1}), so it was expected that investments in exploitation strategies such as hairs and alteration of rhizosphere chemistry by release of organic acid anions or acid phosphatases would return more P to the plants. This was actually the case, as both

genotypes extracted more P (and K, Fig. S4) from the loam plots then was applied as fertilizer (Tab. S3). Our initial hypothesis 'hairs matter at field scale for biomass production and nutrient uptake, in particular for loam as mobility of nutrients is low' was confirmed. For sand the results are explained by higher mobility of fertilizer P during the initial growth stages as compared to loam. For the later growth stages, the results are explained by the required shift to an exploration strategy which altered the C budget (root to shoot ratio) more than the return in P. Differences in root hair development between substrates for the wild-type could provide an alternative explanation for the observed differences between sand and loam. Marin et al. (2020) report longer root hairs for clay loam compared to sandy loam across all barley genotypes investigated. Note that root hair length was not measured in our field experiment, but in the twin laboratory experiment with the same treatments no differences
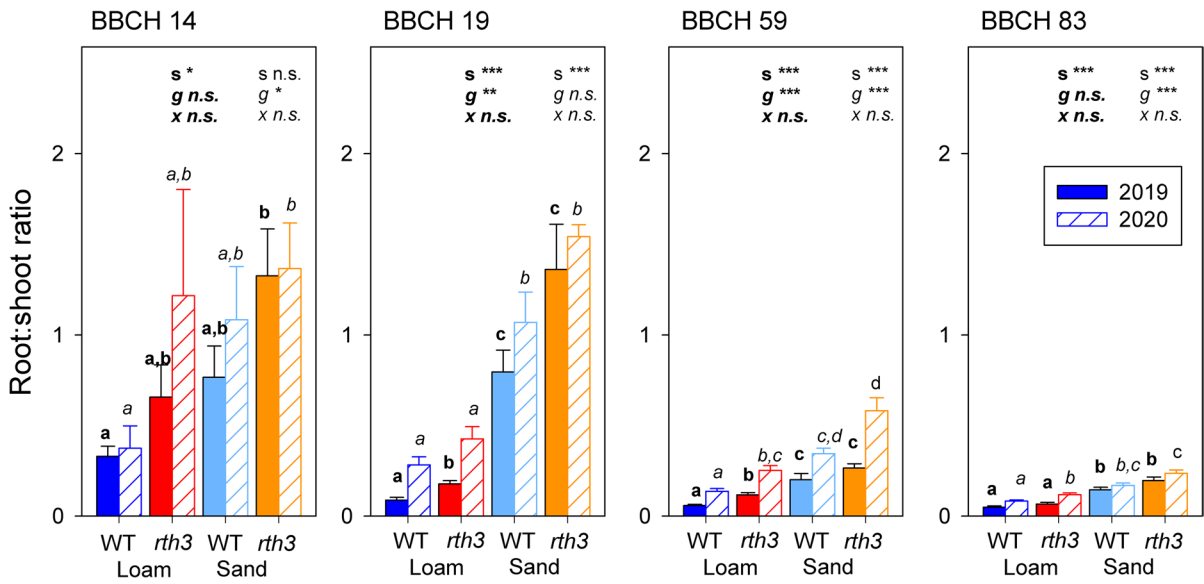
Fig. 9 Impact of substrate (loam, sand) and maize genotype (wild-type—WT, root hair mutant *rth3*–*rth3*) on root to shoot dry weight ratio at different stages of plant development (BBCH 14, BBCH 19, BBCH 59, BBCH 83) in the first (2019) and second (2020) year of soil plot experiment. Statistics: two-factorial ANOVA in conjunction with Tukey's HSD test was conducted for growth stage. Significant effect of factor is denoted by s for substrate, g for genotype and x for interaction. Differences between treatments ($p < 0.05$) are indicated by different lower case letters. Whiskers indicate standard error, n = 6. Bold letters refer to 2019, italic letters to 2020

Fig. 10 Impact of substrate (loam, sand) and maize genotype (wild-type—WT, root hair mutant *rth3*–*rth3*) on water use efficiency at different stages of plant development (BBCH 14, BBCH 19, BBCH 59) in the first (2019) and second (2020) year of soil plot experiment. Note that soil water extraction data are based on field measurement summed up across the soil profile (Jorda et al. submitted) for one replicate of each treatment. For comparison inset provides data for the same treatments in the twin laboratory experiment (SCE) for growth stage BBCH 14 under well-watered conditions (Lippold et al. 2021). WUE is provided with (shoot plus root) and without (shoot) including root dry weight
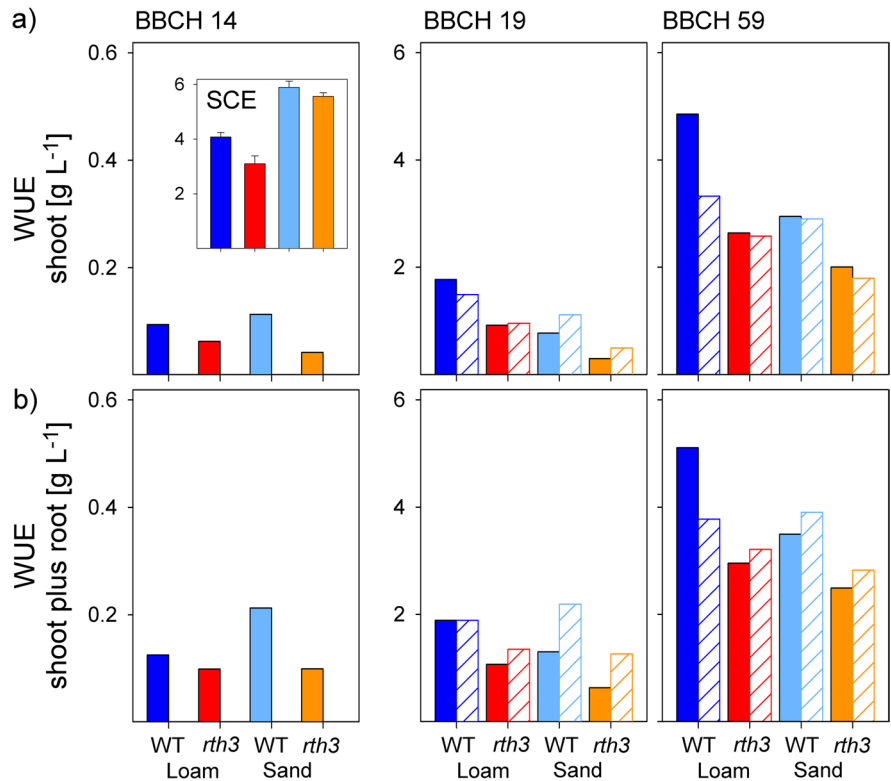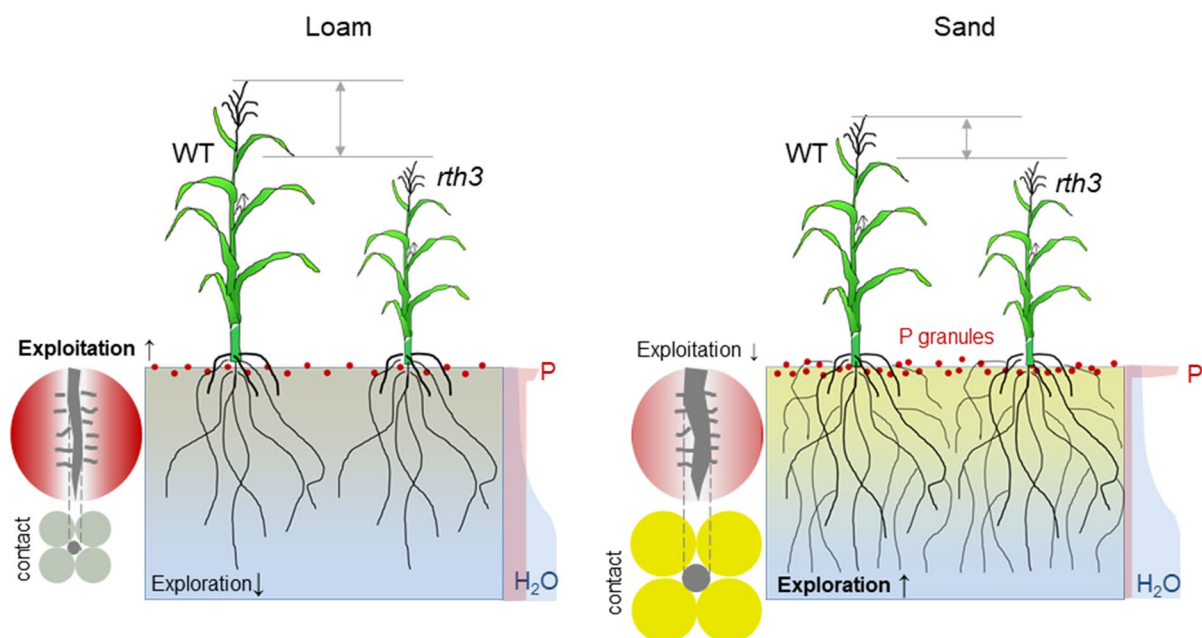
Fig. 11 Graphical summary with schematic representation of the response of shoot and root growth of maize wild type and root hair mutant *rth3* to texture (loam and sand). The genotypes differed in shoot growth, with greater differences in loam than in sand. For root traits, especially root length density and root diameter, the differences between genotypes were small, but texture resulted in a large plasticity. The higher investment in root length density in sand is related to the lower concentration of plant-available nutrients in sand than in loam, which favours exploration over exploitation. Larger root diameters in sand than in loam are more likely explained by the need for soil contact of the roots

in root hair length between the two substrates were observed (Lippold et al. 2021).

Do root hairs contribute to water acquisition by plants, especially under drought stress, and is this effect more important in sandy substrates?

In both years precipitation was exceptionally low compared to the long-term average for the region and hence drought stress developed as the growing season advanced. As described above, up to growth stages BBCH 14 and BBCH 19, differences in shoot growth between genotypes can be explained by the availability of N, P and K. The strong decline in plant available water between BBCH 19 and 59, in particular in the topsoil (Fig. 1), did not only render fertilizer less available in the topsoil, but resulted in visible symptoms of drought stress (leaf rolling) and in an increase of mechanical impedance from 0.5 MPa to > 2.2 MPa (Rosskopf et al. submitted). We observed an earlier onset of drought stress for the wild-type in both years, which was related to larger shoot size and hence water requirement (potential transpiration) at that specific time point. Wild-type in loam suffered particularly strongly from drought stress and did not show any biomass increase past BBCH 59 in 2019. Hence, despite significant differences in shoot biomass development between genotypes the cumulative soil water extraction for the whole growing season, normalized to soil surface area was similar between the wild-type and *rth3* (Jorda et al. submitted). However, when cumulative water extraction is normalised to shoot dry weight, we observed higher water use efficiency of wild-type as compared to the root hair mutant. Higher water use efficiency could be explained by a more sensitive regulation of stomata in response to drought (Benešová et al. 2012) or by a more inefficient use of the assimilated carbon by the root hair mutant. The latter could be related to higher maintenance respiration (Earl et al. 2012) or root exudation (Santangeli et al. personal communication) paired with an already larger investment into roots relative to shoot size. Even when the root biomass itself is accounted for in the calculation

of water use efficiency (Fig. 10b) the discrepancy between genotypes remained and was observed in both years. Based on the literature (Carminati et al. 2017b), hairs were expected to improve root-soil contact and increase the apparent root radius. Both mechanisms would help avoid or delay steep water potential gradients around the roots, thus allowing a longer maintenance of soil water extraction and thus a more thorough utilisation of plant-available soil water. These mechanisms would not be expected to alter water use efficiency per se, because they would increase water uptake and biomass production at the same time. More importantly, the relevance of this bridging effect has only been experimentally proven for barley, but has not been confirmed for maize in dedicated laboratory studies with the same substrates and genotypes (Cai et al. 2021). Simulation of plant water relations for the present field study with Hydrus 1D matched measured results for soil water extraction, transpiration flux and plant water potential without requiring a genotype specific parametrisation of root hydraulic conductivity. The model runs showed that root length density never limited water extraction from the soil at the rate required by the evapotranspiration demand. In relation to shoot size and their transpiration needs, there were always enough roots to exploit soil water down to permanent wilting point (Jorda et al. submitted). Note that the model used for the simulations does not explicitly account for the conductivity/resistance of the rhizosphere.

Currently it cannot be ruled out that the observed differences in water use efficiency between genotypes might be the result of a pleiotropic effect of the *rth3* mutation. Differences in water use efficiency are systematic, as they were not only observed in the field or upon water limitation, but are likewise reported for laboratory experiments under well-watered conditions (Fig. 10). Further studies addressing constitutive or inducible differences in stomata number and photosynthetic water use efficiency are currently conducted. Plasma membrane aquaporin expression in roots is not affected by maize genotype, and the changes in transcript abundancies due to substrate and drought stress at BBCH59 are conserved between the wild-type and *rth3* roots (Ganther et al., submitted). Alternatively, if root hairs function as sensors for drought stress as recently suggested by Kwasniewski et al. (2016), this could also explain the observed differences.

Is the root hair mutant rth3 showing plasticity in other root traits to compensate for the lower surface? What is the relevance rhizodeposition /carbon partitioning?

Root traits

Root dry weight and related root to shoot ratio development over time as well as absolute values were well within the range of values reported by Amos and Walters (2006) and Ordóñez et al. (2020) reviewing published data for maize (n = 45 and 125, respectively). Maximum root biomass production is expected at tassel emergence (BBCH 59) or shortly thereafter and root to shoot ratio decreases as plants grow and remains nearly constant during the reproductive growth stages (Amos and Walters 2006). This was the case for our root growth data (Fig. 5) and root to shoot ratios (Fig. 9) in both years. The selection of constant distance of 10 cm to the base of the plant for all sampling time points was a suitable compromise between overestimation of early root growth when sampling under the plant and overestimation for the later growth stages when sampling at mid-distance between the rows (Gajri et al. 1994). It proved important that sampling was conducted in three depth intervals down to 60 cm as the general assumption of root mass or length decreasing with depth, with the majority of roots located in the top 20 to 30 cm ((Amos and Walters 2006) and citations therein) did not hold true under the encountered environmental conditions (drought, homogeneous substrate).

Root to shoot ratio

Our values at BBCH 59 and 83 are within the data range of 0.02 to 0.4 for maize derived from a meta-analysis of 125 datasets provided by Ordóñez et al. (2020). This paper indicates that maize as compared to soybean has a very high plasticity in root to shoot ratio and that shoot biomass cannot be used to estimate root biomass. This applies even more to the estimation of root length. This plasticity is reflected in significant differences in root to shoot ratios between our treatments and the different impact of drivers (substrate, genotype) on shoot biomass (Fig. 3) versus root biomass (not shown) or root length density (Fig. 5). The higher root to shoot ratio for the root

hair mutant can be regarded as a compensation mechanism as stated in hypotheses 3.

Root length density

The mean values for root length density (n=17) reported for the reproductive phase of 1.8 ($\pm$0.15) cm cm^{-3} (Amos and Walters 2006; Ordóñez et al. 2020) is higher than the values we observed for loam, but lower than those measured for sand. Root length density depth profiles serve as a proxy for root architecture at field scale. For the field, as in the corresponding laboratory experiments (Lippold et al. 2021), no change in root length density profiles in response to the lack of root hairs could be found. This is in contrast to what has been suggested for other root hairless maize (rth2, (Klamer et al. 2019)) or barley mutants (brb, (Dodd and Diatloff 2016). The substrate, in turn, caused a pronounced and unexpected difference in root length density, which was accompanied by a shift in root length density across root diameter classes (Fig. S5). The larger root length densities for sand can in part be explained by the lower content in plant available nutrients. This is valid except for the surface where fertilizer has been applied. Progressive soil drying shifted root growth to larger soil depths. The fact that this shift was more pronounced for loam than sand might again be related to the more uneven nutrient distribution in sand. Higher root lengths in the coarser substrate were also observed by Marin et al. (2020) for barley comparing sandy loam and clay loam.

Root diameter

The higher share of root length in the smallest root diameter class (0–100 µm) for loam as compared to sand is reflected in the lower root mean diameters (Fig. 6). Larger share of fine roots is typically reported for nutrient rich patches in heterogeneous soils or for substrates with low availability of nutrients in general (Hodge 2004; Strock et al. 2018; Vejchasarn et al. 2016). However, in the present experiment nutrients were homogeneously distributed except for the top centimetres affected by surface applied fertilizer. Plant available nutrient concentrations below the fertilizer affected surface were higher in loam than in sand. Similar differences in root diameters between the substrates have also been observed

for the twin laboratory experiment for which fertilizer was homogeneously mixed into the substrate and similar amounts of plant available nutrients were present (Lippold et al. 2021). Doubling the amount of fertilizer in the laboratory experiments had no impact on root diameters (data not shown). Hence it is unlikely that nutrients are responsible for the observed differences in diameter. Increases in root diameters have most frequently been observed in response to increases in soil penetration resistance (Bengough et al. 2006; Correa et al. 2019). For the laboratory experiment penetration resistance was in the range of 0.1 to 0.5 MPa for sand and loam respectively (Rosskopf et al. 2021). For samples collected from the field values ranged from 0.5 to 2.2 MPa with water potential decreasing from -3 to -50 kPa with no significant differences between sand and loam in neither of the two depths investigated (Rosskopf et al. submitted). Not only there was no difference in penetration resistance between the substrates within the measured range of soil matric potential, below which the values only fell after BBCH 19 in 2019, but there was also no associated decrease in root growth (root length), which is typically observed when soil penetration resistance affects roots (Correa et al. 2019).

Arbuscular mycorrhiza (AM) formation

We expected that the rth3 plants would show higher colonisation levels, especially as mycorrhiza formation can actually compensate for the loss of root hairs in the rth3 mutant. By implementing an AMF inoculation experiment, Ma et al. (2021) showed that the positive effects of mycorrhiza formation on plant growth and P acquisition in rth3 were 1.5- to 3.9-fold greater than in the wild-type, and AMF colonisation complemented growth and P acquisition defects of the mutant. Interestingly, mycorrhizas also effectively substituted root hairs in P uptake in barley (Jakobsen et al. 2005). Our data suggest that either the rth3 maize was not P limited but instead limited in mineral nutrients whose uptake is not dependent on mycorrhizal fungi (Kothari et al. 1990; Liu et al. 2000), or it was not able to allocate enough carbon to the mycorrhizal fungi to support a higher colonisation of the roots than observed (Fellbaum et al. 2012), and support the mycorrhiza dependent nutrient uptake. In general, availability of P in the soil in part determines how maize is colonised and how it responds

to mycorrhiza colonization under field conditions. In this field experiment, the low levels of available P should support AM colonisation of maize roots.

In summary, we cannot confirm our hypothesis that *rth3* shows plasticity to compensate for the lower surface induced by the lack of root hairs, apart from a higher investment in root growth relative to shoot growth (Fig. 11). However, both genotypes did show strong plasticity of root traits in response to substrate. While higher root length density in sand as compared to loam is probably brought about by the lower content of plant available nutrients (Gao et al. 2015; Hodge 2004), this does not explain the increase in mean root diameter observed for sand. Higher root length density combined with an increase in diameter and a smaller share of aerenchyma added up to a substantially larger input of carbon for sand as compared to loam which is expected to impact carbon sequestration over the years. Interestingly the observation of slower root degradation in sand is in line with observations from a laboratory experiment on biopore recycling for which X-ray CT revealed likewise that roots in loam were completely degraded after 78 days while still present in sand after 216 days (Phalempin et al. 2022).

What explains the texture related differences in root traits beyond differences in nutrient supply?

It is a unique feature of our experiment based on artificially established soil profiles, that we can compare the effect of texture on root growth in the field under identical environmental conditions. Other studies comparing different soil types or texture had to do so by establishing experiments at different sites or at least different areas within large sites (Chassot et al. 2001; Dwyer et al. 1988; Marin et al. 2020; Qin et al. 2005; Sene et al. 1985).

There is a number of studies which investigated root traits for soils differing in texture, however, most of these studies did either (i) not report information on root diameter, (ii) could explain observed increases in root diameter by increasing mechanical resistance/compaction, or (iii) could not separate the effect of texture from differences in environmental conditions and management (precipitation, temperature, nutrient availability, soil cultivation) (Table S4 and citations therein). Surprisingly few studies have addressed

explicitly the impact of particle size distribution (Anderson et al. 2007; Rogers et al. 2016; Warnaars and Eavis 1972) or the shape of particles (Lipiec et al. 2016) on root diameter. Rogers et al. (2016) found no consistent pattern among different rice genotypes; some did show an increase in root diameter with coarser substrate texture. Warnaars and Eavis (1972) found a decrease in specific root length, used as a surrogate for root diameter, with increasing particle sizes and likewise Anderson et al. (2007) reported the share of fine roots to decrease with increase in soil sand fraction. Larger root diameters in sand as compared to loam were observed throughout all growth stages and depths in 2019 when plants grew into the homogenized soil structure for the first time. For this reason it is unlikely that differences in root diameters can be explained by substrate specific shifts between root types (brace, crown, seminal root axis and respective laterals). In addition, the differences in diameter corresponded to different ratios or cortex width to vascular bundle width measured for main root axis. This would not be expected if the differences would be due to a larger share of fine lateral roots. Coarser particles or aggregates will show a lower contact area with a smooth surface such as the root surface as compared to fine particles. This was demonstrated by Schmidt et al. (2012) for maize roots growing in aggregate fractions of 4–2, 2–1, 1–0.5 and < 0.5 mm. Reduced root-soil contact is potentially unfavourable for nutrient and water uptake and might trigger adaption mechanisms to compensate for this. Increasing root diameter could be such an adaption mechanism but one associated with high carbon costs (Lynch and Ho 2005). Currently the mechano-sensing of roots is poorly understood (Dupuy et al. 2018; Monshausen and Gilroy 2009). Roots can show responses to touch or impermeable mechanical barriers by changes in cell division patterns, growth direction, cell differentiation and gene expression. Changes in pressure at the plant cell surface are signalled by mechano-sensitive ion channels and kinases to the microtubule cytoskeleton, and lead to altered cytoskeletal architecture and reinforcement of the cell wall (Hamilton et al. 2015; Tang et al. 2021).

Dupuy et al. (2018) recently suggested re-visiting micromechanics of rooting development in soil by considering recent advances in granular

matter physics. They pointed out that the 'classical mechanics of continua' is ill-equipped to explain the links between soil heterogeneity and stochasticity of plant development. They argue that this is of particular relevance for medium grained soils; here roots can displace individual particles from the soil, but the forces exerted by each of the particles can also influence the course of root development. The latter is not reflected in the measurement of mechanical impedance (Rosskopf et al. 2021). Crucial for this type of concept is the size aspect ratio between root's diameter and typical length scale of soil heterogeneity (particle size) (Kolb et al. 2017). The increase of root diameter observed in sand can be interpreted as an attempt to maintain an optimal size aspect ratio.

The root phene 'increased root diameter' can be induced by ethylene as such, i.e. even in systems without soil or pressure (Sarquis et al. 1991). Pandey et al. (2021) recently concluded from their study that ethylene acts as an early warning signal for roots to avoid compacted soil and that the mechanism is related to altered gas diffusion in the rhizosphere. Hence the questions arise whether ethylene is also the signalling substance in our systems, and which mechanisms may result in increased ethylene concentrations in the rhizosphere. Root-soil contact (Schmidt et al. 2012), bulk density gradients in the rhizosphere (Phalempin et al. 2021) as well as gas diffusion per se differ between sand and loam due to differences in porosity and soil water content.

Sensing of contact has been demonstrated for root tips (Massa and Gilroy 2003), and is very likely related to root cap-dependent ethylene emission (Dreyer and Edelmann 2018). Sensing of contact points along the root surface could thus also be related to ethylene production and or distribution and release. Hence, as an alternative to differences in ethylene diffusion between substrates, we suggest that ethylene production might be triggered specifically in sand related to sensing of contact. The role of hairs in this respect is unclear. They might be involved in sensing contact, but they have also been reported to respond to increased ethylene concentrations, i.e. increased root hair length and numbers (Feng et al. 2017).

Conclusion and outlook

Genotypes differed in shoot growth with differences being larger in loam than sand in line with hypothesis 1 (Fig. 11). For the early growth stages, as long as topsoil was moist, exploitation strategy related to the presence of hairs might have been successful to tap the applied fertilizer. For later growth stages, as drought developed, roots had to explore the entire soil volume in particular for sand, being overall low in plant available nutrients. Root architecture was shaped primarily by the need to access nutrients with increasing drought progressively altering the volume which could be successfully explored for nutrients. Obviously exploitation strategy related to hairs ranked second under these conditions, in particular in sand. This explains the high root length density in sand associated with high root to shoot ratio. The larger root diameter in sand than in loam, which was also observed in twin laboratory experiments under well-water conditions for early growth stages was not related to the differences in mechanical resistance between substrates. We hypothesize that this is a general phenomenon in coarse textured substrates related to the need of optimising root-soil contact. The underlying mechanisms should be explored in future dedicated experiments. Higher root length density along with larger root diameters resulted in higher carbon input in sand than in loam. The impact of altered carbon budgets on microbial community composition and microbial activity as well as soil structure formation and stability is under investigation.

Author Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Doris Vetterlein, Steffen Schlüter and Mika Tarkka. The first draft of the manuscript was written by Doris Vetterlein and all authors commented

Declarations

Competing Interest The authors have no relevant financial or non-financial interests to disclose

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