Root trait plasticity and plant nutrient acquisition in phosphorus limited soil

Amit Kumar[1,2,3]*, Muhammad Shahbaz[4,5], Manisha Koirala[2], Evgenia Blagodatskaya[6,7], Sabine Julia Seidel[8], Yakov Kuzyakov[2,9], and **Johanna Pausch[1]**

[1] Department of Agroecology, University of Bayreuth, Universitätsstr. 30, 95440 Bayreuth, Germany
[2] Department of Agricultural Soil Science and Department of Soil Science of Temperature Ecosystems, Georg-August University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany
[3] Current address: Chair of Ecosystem Functioning and Services, Institute of Ecology, Leuphana University of Lüneburg, Universitätsallee 1, 21335, Lüneburg, Germany
[4] Department of Soil and Environment, Swedish University of Agricultural Sciences, P.O. Box 7014, 75007, Uppsala, Sweden
[5] Centre for Environmental and Climate Research, Lund University, 22362 Lund, Sweden
[6] Department of Soil Ecology, Helmholtz Centre for Environmental Research—UFZ, Halle (Saale), Germany
[7] Agro-Technology Institute, RUDN University, Moscow, Russia
[8] Institute of Crop Science and Resource Conservation (INRES), University of Bonn, Katzenburgweg 5, 53115, Bonn, Germany
[9] Institute of Environmental Sciences, Kazan Federal University, 420049 Kazan, Russia

Abstract

To overcome soil nutrient limitation, many plants have developed complex nutrient acquisition strategies including altering root morphology, root hair formation or colonization by arbuscular mycorrhizal fungi (AMF). The interactions of these strategies and their plasticity are, however, affected by soil nutrient status throughout plant growth. Such plasticity is decisive for plant phosphorus (P) acquisition in P-limited soils. We investigated the P acquisition strategies and their plasticity of two maize genotypes characterized by the presence or absence of root hairs. We hypothesized that in the absence of root hairs plant growth is facilitated by traits with complementary functions, *e.g.*, by higher root mycorrhizal colonization. This dependence on complementary traits will decrease in P fertilized soils. At early growth stages, root hairs are of little benefit for nutrient uptake. Regardless of the presence or absence of root hairs, plants produced average root biomass of 0.14 g per plant and exhibited 23% root mycorrhizal colonization. At later growth stages of maize, contrasting mechanisms with functional complementarity explained similar plant biomass production under P limitation: the presence of root hairs *versus* higher root mycorrhizal colonization (67%) favored by increased fine root diameter in absence of root hairs. P fertilization decreased the dependence of plant on specific root traits for nutrient acquisition. Through root trait plasticity, plants can minimize trade-offs for developing and maintaining functional traits, while increasing the benefit in terms of nutrient acquisition and plant growth. The present study highlights the plasticity of functional root traits for efficient nutrient acquisition strategies in agricultural systems with low nutrient availability.

Supporting Information available online

Key words: arbuscular mycorrhizal colonization / nutrient acquisition / root hairs / root morphology / *roothairless3* mutant / root traits

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1 Introduction

Phosphorus (P) is one of the most growth-limiting macro-nutrients, and root systems display diverse morphological and physiological strategies to enhance its uptake at low and heterogeneous P availability in soil (*Lambers* et al., 2006; 2013). For example, under P limitation, primary root growth is suppressed and the root architecture changes to a shallower root system featuring more lateral roots and increased root hair density. This enables the roots to explore a larger soil volume (*Péret* et al., 2011). Root physiological strategies include exudation of various organic anions and release of extracellular phosphatases to mobilize the otherwise unavailable P in the rhizosphere (*Lambers* et al., 2011; *Richardson* et al., 2011).

An important root morphological trait, root hairs (single-cell extension of epidermal cells), contribute up to 80% to plant P uptake by increasing the root surface area and, hence, the contact space between root and soil for absorption (*Jungk*, 2001; *Jakobsen* et al., 2005; *Li* et al., 2014). Increased surface area with root hairs, in turn, contributes to the release of root-derived organics and phosphatases which subsequently increase P acquisition. For example, barley roots possessing hairs radially extended the rhizosphere by three times as compared to the mutant lacking root hairs (*Holz* et al., 2018). Another important root functional trait with complementary function (increased P acquisition) is root colonization by

* Correspondence: A. Kumar; e-mail: akumar4@gwdg.de; kumar@leuphana.de

arbuscular mycorrhizal fungi (AMF) (*Munyanziza* et al., 1997). Plants trade carbon (C) to AMF for in exchange of nutrients. The extraradical mycelia of AMF may also enter the very fine soil pores, thereby increasing the nutrient uptake (*Khalvati* et al., 2005). Here, we emphasize the rationale to consider root colonization with AMF as a 'trait' because approximately 18% of angiosperm species (*e.g*., members of Brassicaceae, Chenopodiaceae, Cyperaceae, Zygophyllaeae families) lack any symbiotic associations with fungi and about 30% of angiosperm species establish other (than AMF) types of mycorrhizal associations (*Brundrett*, 2002; *Brundrett* and *Tedersoo*, 2019). Given that a fungus is considered as mycorrhizal only after its successful interaction with roots to form a specific type of symbiotic association, the consideration of root colonization by mycorrhizal fungi as a trait is highly feasible. Mycorrhizal symbiosis may trigger various adaptive strategies into plant such as changes in the root-to-shoot ratio (*Veresoglou* et al., 2012), root architecture and longevity (*Hooker* and *Atkinson*, 1996), root length (*Camenzind* et al., 2016), and root diameter (*Comas* et al., 2014). Such allometric changes are plant-species-specific and depend on the duration of the experiments as well as on the identity of the plant and its fungal partner identities (*Veresoglou* et al., 2012). Much of this information is derived from plant phylogeny by determining changes in root morphological and architectural traits using phylogenetically independent contrasts (*Comas* et al., 2014). Accordingly, an in-depth understanding requires empirical evidence. The notion that plants colonized with AMF increase their root diameter has been forwarded (*Brundrett* and *Tedersoo*, 2019), but explicit observations are missing. Moreover, most studies on plant-mycorrhizal interactions have focused on one plant growth stage, almost ignoring the dynamics of nutrient acquisition related to plant phenology. Such temporal changes in plant microbial interactions are defined as a 'missing factor' and highlight the necessity of incorporating those in future ecological studies (*Schofield* et al., 2018). Interactions between roots and microorganisms are established at early plant growth stages. At later stages, such interactions gain importance due to plant-growth-associated changes in root morphology, soil properties (*Philippot* et al., 2013; *Wen* et al., 2017), and variation in rhizodeposits quality and quantity (*Chaparro* et al., 2014). Intense competition between plants and soil microorganisms for limited nutrients also play a role (*Kuzyakov* and *Xu*, 2013). For example, root exudation changes with plant growth stage (*Chaparro* et al., 2014) and results in altered enzyme activities in rooted soil (*Kumar* et al., 2018). Throughout the growth period, plants adapt their strategies to maximize nutrient uptake, but no explicit relationship between specific root traits and nutrient acquisition has been determined (*Chen* et al., 2016). Such strategies are plant-species-specific and the causes of variations are not conclusive. For example, increased root hair length and density in *Brachypodium* cultivars were insufficient to increase plant P uptake and must be combined with other root traits to enhance P acquisition (*Zhang* et al., 2018).

The present study focuses on the root trait plasticity for plant P acquisition in P-limited soil and on the response of these traits to increased P availability *via* P fertilization. To this end, maize with (wild type, WT) and without root hairs (*roothairless3* mutant, *rth3*) were grown for 64 days in a climate chamber under controlled environmental conditions. Subsets of both genotypes were fertilized with KH_2PO_4. We hypothesized that (1) in P-limited soils and in absence of root hairs (key trait for plant nutrient acquisition), plant growth is maintained by shifting the root traits to attract colonization by AMF for P acquisition, whereas (2) with P fertilization, plants become less dependent on specific root traits for P uptake. This study provides the opportunity to understand the plants' P uptake strategies through plasticity in root traits at three plant growth stages (tillering, stem extension, maize heading).

2 Material and methods

2.1 Experimental setup

The soil was sampled from the long-term fertilizer experiment Dikopshof of the University of Bonn (50°48′17′′ N, 6°57′17′′ E). The soil was collected from the topsoil (0–20 cm) of an unfertilized control plot (without any fertilizer application since 1942). The soil was classified as a Luvisol derived from loess above sand. The soil texture near the unfertilized plot is 11.8% sand, 71.2% silt, and 17.0% clay (German clay, silt and sand particle-size ranges, DIN ISO 11277) with the following properties: total C (7.8 ± 0.02 g C kg^{-1} soil), total N (0.74 ± 0.01 g N kg^{-1} soil), C to N ratio (10.5 ± 0.02), calcium-acetate-lactate extractable P (23.2 ± 0.7 mg P kg^{-1} soil), and soil pH 6.48. Field moist soil was sieved (2 mm) and 1.5 kg dry weight equivalent was filled in polyvinyl chloride (PVC) pots (KG-tubes, height 20 cm, diameter 10 cm). All soil-filled pots were pre-incubated in a growth chamber for 3 days before sowing.

Seeds of a maize (*Zea mays* L.) wild type (WT) and a mutant lacking root hairs (*roothairless3*; *rth3*) (*Hochholdinger* et al., 2008) were surface sterilized with 10% H_2O_2 for 3 min, washed 5 times with distilled water, and germinated on moist filter paper in petri plates in the dark for 5 days. After germination, seedlings were transferred to PVC pots containing soil sampled from Dikopshof (1 seedling per pot) and grown under controlled environmental conditions in a climate-chamber with 16 h : 8 h light : dark rhythm with mid-day and night temperatures of 25°C and 15°C, respectively, and a light intensity of approximately 600 μmol m^{-2} s^{-1}. After establishment of seedlings, all pots were fertilized with mineral N (KNO_3, at the rate of 120 kg N ha^{-1}) to avoid soil N limitation, whereas mineral P was added to the half of total number of pots. Half of the pots received mineral P (KH_2PO_4, at the rate of 60 kg P ha^{-1}). The soil water content was checked every alternative day and maintained at 70% water holding capacity (WHC) with distilled water throughout the experimental period. To account for effects of plant phenological stage on rhizosphere processes, samples were taken 30 days after planting (DAP), 45 DAP, and 64 DAP constituting tillering, stem extension, and maize heading stage, respectively.

2.2 Harvesting

At each harvesting time, four replicates of both genotypes (WT and *rth3*) with or without P fertilization were destructively

harvested. Maize shoots were cut at the base. The main root system was carefully removed after pulling out the soil from the pot. Roots were picked with tweezers from each pot for a specified time period (15 min). A subsample of roots was collected for measurements of arbuscular mycorrhizal colonization (see description below). All roots were scanned with an EPSON (PERFECTION^{TM} V700 PHOTO) scanner and root length density, fine roots, and average root diameter of fine roots (< 2 mm) were determined using WinRHIZO software (Regents Instruments Inc., Quebec, Canada). Afterwards, roots and shoots were freeze-dried and ball-milled to powder for total C, N, and P analyses. The total P content in plant tissues and soil was measured using an inductively coupled plasma-atomic emission spectrometer (iCAP 6300 Duo VIEW ICP Spectrometer, Thermo Fischer Scientific GmbH, Dreieich, Germany). C and N contents were measured using an elemental analyzer 2000 (Thermo Fischer Scientific, Cambridge, UK).

2.3 Root colonization by arbuscular mycorrhizal fungi

Root colonization by AMF was measured after staining the roots with blue ink in lacto-phenol with modifications (*Vierheilig* et al., 1998). Briefly, fine roots (< 2 mm) were collected manually with tweezers for 2 min. Fine roots were cut into 1 cm segments and washed with distilled water. Root segments were cleared in 2.5% KOH at 90°C for 1 h. Thereafter, root segments were washed in distilled water to remove access KOH and treated with 3% H_2O_2 for 30 min at room temperature. Afterwards, the root segments were washed again with distilled water and stained with ink for 2 min. Root mycorrhizal colonization was observed at 10 × 40 magnification under a light microscope (Axionplan, Zeiss, Germany) and the percentage of mycorrhizal colonization was counted using the grid-line intersection method (*Giovannetti* and *Mosse*, 1980). A total of 108 intersects were inspected per plant.

2.4 Phosphomonoesterase activity in soil

Phosphomonoesterase activity was measured with fluorogenically-labeled artificial substrate (4-methylumbelliferylephosphate; MUB) (*Marx* et al., 2001). For this, soil suspension was prepared with 50 mL autoclaved water followed by 2 min low-energy sonication (50 Js^{-1}). Afterwards, an aliquot of 50 μL of soil suspension and 50 μL of MES ($C_6H_{13}NO_4S$-$Na_{0.5}$) buffer (pH 6.5) was added to each well, followed by 100 μL of substrate solution of 4-methylumbelliferyl-phosphate into a black 96-well microplate (PureGradeTM, KG, Wertheim, Germany). The microplate was gently shaken and measurements were taken fluorometrically (excitation 360 nm; emission 450 nm) at 0, 0.5, 1, and 2 h after substrate addition with a micro-plate reader (Victor3 1420-050 Multilabel Counter, PerkinElmer, USA). Amounts of MUB cleaved during the reaction were obtained by converting the fluorescence values using specific standards. MUB cleaved in nmol per g soil dry weight per h (nmol MUB cleaved g^{-1} dry soil h^{-1}) was used to express enzyme activity.

2.5 Statistics

The experiment was conducted with 4 replicates for each treatment at each harvesting time yielding a total of 48 experimental units. The values presented in figures are means ± standard errors of means (± SEM). All statistical analyses were performed in R environment (*R Core Team*, 2018) and graphs were made with 'ggpot2' library (*Wickham*, 2016). We performed two factors ANOVA (fixed factors: maize genotype and P fertilization) for plant biomass (shoot and root biomass), root colonization by AMF, average fine root diameter, as well as for plant N and P uptake at each growth stage and fitted linear models using *lm* function. We plotted fitted and residual values of the model to check for any patterns in our data. Moreover, we also scanned our data with *dotchart* function to check for the presence for outliers. Significant ANOVA results were further analyzed with Tukey's post-hoc test for significant differences using *lsmeans* and *cld* functions (lsmeans; *Lenth*, 2016) for which we used the original linear model. Correlation analyses were performed with *stat_cor* function (ggpurb; *Kassambara*, 2018).

3 Results

3.1 Plant biomass

Total plant biomass (root and shoot biomass) increased from the tillering to maize heading stage for both (wild type and *rth3* mutant) genotypes (Fig. 1a, b). The *rth3* mutant maize (completely lacking root hairs) produced almost similar biomass (shoot and root) as the WT maize (possessing root hairs). Phosphorus fertilization resulted in higher biomass production at each growth stage and increased the biomass by 38% in the mutant (*rth3*) and by 43% in the wild type (WT) at maize heading.

3.2 Root morphology, mycorrhizal colonization, and phosphomonoesterase activity

The average fine root diameter (AFRD; in mm) increased with P fertilization for the WT maize during tillering and stem elongation stages (Fig. 2a). This increase was up to 21% at tillering and 10% at the stem elongation stage. There was a trend for an increase in AFRD for the *rth3* maize with P fertilization up to 8%, 3%, and 10% at tillering, stem elongation, and maize heading, respectively, as compared with no P addition. Comparing the two genotypes, the hairless mutant always had a greater AFRD than the WT maize. The AFRD of unfertilized *rth3* type increased by 16%, 15%, and 20% compared to WT at tillering, stem elongation, and maize heading, respectively. Moreover, the AFRD of the hairless mutant (*rth3)* increased gradually with plant growth stage (0.39 ± 0.01 mm at tillering, 0.45 ± 0.02 mm at stem elongation, and 0.47 ± 0.02 mm at maize heading) (Fig. 2a). Root colonization by AMF decreased with P fertilization by 19%, 24%, and 16% in WT and 17%, 27%, and 19% in *rth3* maize roots at tillering, stem elongation, and heading stage, respectively (Fig. 2b). The AMF colonization increased in both WT and *rth3* with plant growth stage. This increase, however, was more pronounced in *rth3* without P fertilization. The root colonization

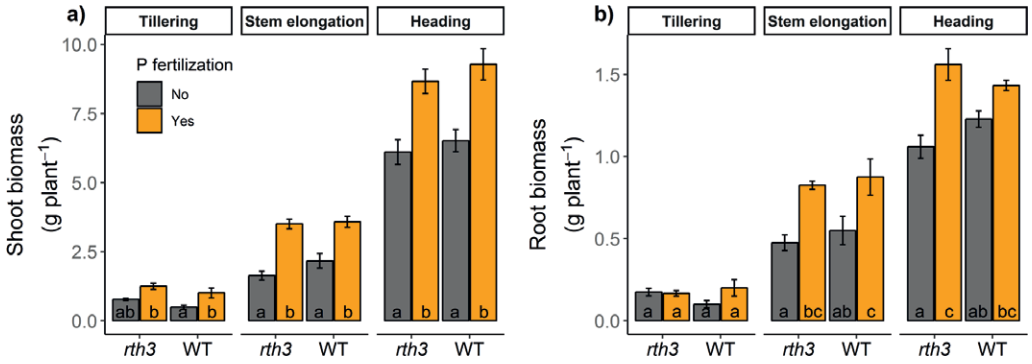

Figure 1: (a) Shoot and (b) root biomass (g plant^{-1} ± SE) of maize plants with (wild type: WT) and without root hairs (mutant: *rth3*). Letters indicate significant differences (two factor ANOVA, Tukey's post-hoc, $p < 0.05$) between genotypes and P fertilization at each growth stage: tillering, stem elongation, and heading.

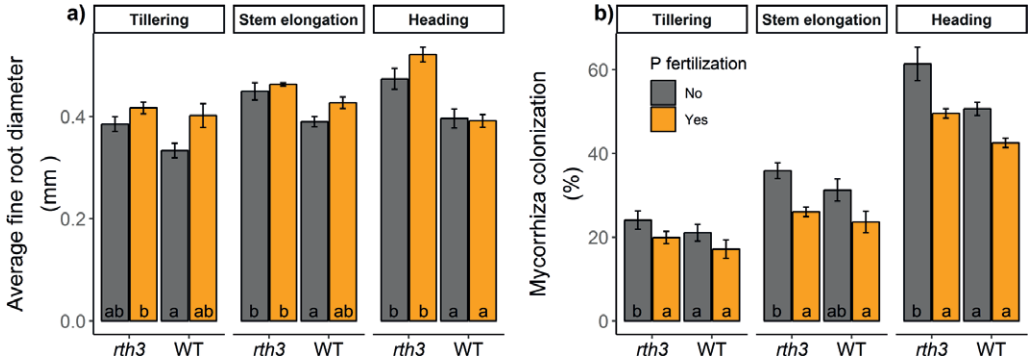

Figure 2: (a) Average fine root diameter (mm ± SE) and (b) root mycorrhiza colonization (%) of maize plants with (wild type: WT) and without root hairs (mutant: *rth3*). Letters indicate significant differences (two factor ANOVA, Tukey's post-hoc, $p < 0.05$) between genotypes and P fertilization at each growth stage: tillering, stem elongation, and heading.

by AMF increased by 14%, 15%, and 21% at tillering, stem elongation, and heading, respectively, in unfertilized *rth3 versus* WT maize roots (Fig. 2b). Root colonization by AMF and AFRD correlated (r = 0.55, $p < 0.01$) positively for *rth3*, but not in WT (Fig. 3a). Potential activity of phosphomonoesterase (PHO) enzyme increased during plant growth in both WT and *rth3* maize planted soil. At tillering and stem elongation, PHO activity was independent of P fertilization, but the activity at maize heading stage decreased by 18% and 20% in WT and *rth3* maize soil, respectively (compared with unfertilized soil; Supplementary Tab. 1). Moreover, there was a significant correlation between AFRD and PHO activity in *rth3* (r = 0.67, $p < 0.01$) but not in WT (Fig. 3b).

3.3 Phosphorus and nitrogen uptake

Shoot P uptake, reported as total P content at each sampling, increased with growth stage in both WT (from 0.71 ± 0.08 mg P plant^{-1} to 9.30 ± 0.63 mg P plant^{-1} from tillering to heading stage, respectively) and *rth3* (from 0.94 ± 0.08 mg P plant^{-1} to 8.78 ± 0.47 mg P plant^{-1} from tillering to heading stage, respectively) maize. Compared with unfertilized maize, P fertilization increased the shoot P uptake by 311%, 164%, and 61% in WT maize and by 255%, 223%, and 67% in *rth3* maize at tillering, stem elongation, and heading stage, respectively (Fig. 4a). Shoot P uptake was similar between the genotypes (WT and *rth3*) either with or without P fertilization at every maize growth stage. A similar response of P fertilization and genotypes was observed for the root P uptake (except for the non-significant effect at tillering) (Supplementary Tab. 1). Plant N uptake showed a similar pattern to that of P uptake with respect to P fertilization and genotypes, although the strength of the P fertilization effect was reduced. Similar to the P uptake, shoot N uptake also increased with plant growth stage in both genotypes (18 ± 2.2 mg N plant^{-1}, 32 ± 4.3 mg N plant^{-1}, and 76 ± 2.8 mg N plant^{-1} in WT and 24 ± 2.7 mg N plant^{-1}, 32 ± 3.1 mg N plant^{-1}, and 67 2.4 mg N plant^{-1} in *rth3* maize at tillering, stem elongation, and heading stage, respectively) (Fig. 4b). Phosphorus fertilization increased the shoot N uptake by 78%, 57%, and 13% in WT and 76%, 63%, and 15% in *rth3* maize.

4 Discussion

4.1 Plant biomass and root mycorrhizal colonization

Plant biomass increased with P fertilization as often observed for grasses (*Sundqvist* et al., 2014; *Haines* et al., 2015), other agricultural crops (*Gahoonia* et al., 1999; *Bakhshandeh* et al., 2017), and trees (*Davidson* et al., 2004). Phosphorus ferti-

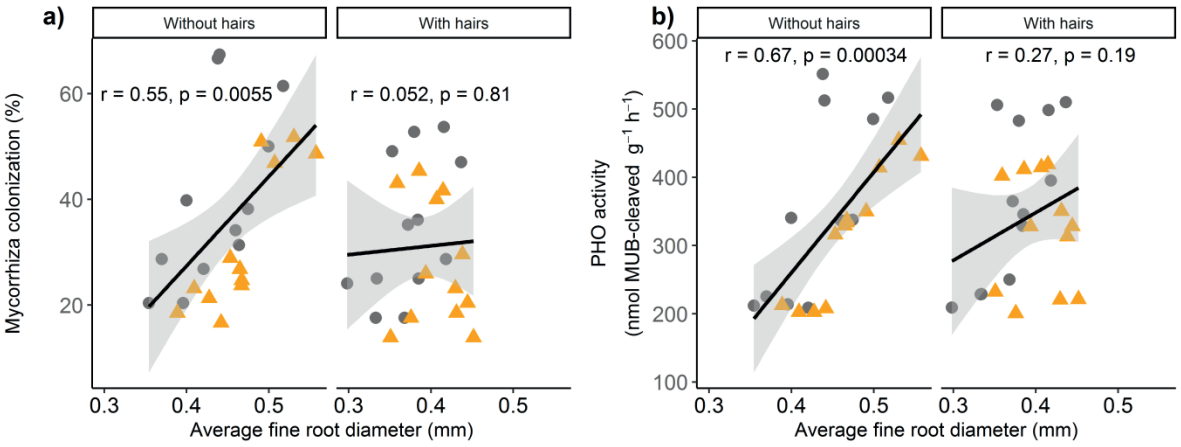

Figure 3: (a) Correlation between average fine root diameter (mm) root AMF colonization and (b) average fine root diameter and PHO activity for maize with and without root hairs including all three growth stages. Orange (triangle) and grey (circle) symbols are with and without P fertilization, respectively.

lization increased the net P and N uptake (Fig. 4), resulting in higher photosynthetic activity and higher biomass production. A similar increase in the biomass (by 38%) of different maize cultivars with P fertilization was observed by *Bukvić* et al. (2003), however, it should be noted that the overall plant biomass per pot may not represent field conditions.

The stronger root colonization by AMF in the hairless mutant indicated that the lack of one functional root trait (here root hairs) caused shifts to other traits (here mycorrhiza) with complementary functions. These traits may, however, be more C cost intensive and their development may be down-regulated if the respective function is not required. In soils with high P availability, for instance, the importance of functional traits for P uptake is reduced (lower colonization by AMF) (Fig. 2b). Depending on plant identity and fungal partner, about 4–30% of net photo-assimilated products transferred to mycorrhizal fungi (*Smith* and *Smith*, 1990) may not benefit the plant directly when sufficient P is available for plant growth. In soils with high P availability, the fungal partner does not benefit P acquisition by plants yet receives a significant amount of photo-assimilated products (*Graham* and *Eissenstat*, 1998; *Treseder* and *Allen*, 2002). This might provoke negative feedbacks to plants (*Kiers* et al., 2002). In contrast, when plant growth is limited by P, root colonization by AMF alleviates such negative responses.

4.2 Effect of phosphorus fertilization on mycorrhizal colonization and phosphomonoesterase activity

Phosphorus fertilization increased the total plant biomass (Fig. 1) but reduced root colonization by AMF as compared to unfertilized soil (Fig. 2b). This highlights the importance of mycorrhizal symbiosis for plant P acquisition. Moreover, when plant growth is not nutrient limited, the higher trade-offs for establishing and maintaining mycorrhizal symbiosis exceeds their benefits to plants (*Carbonnel* and *Gutjahr*, 2014; *Zhang* et al., 2018) which might have resulted in reduced root colonization with P fertilization. The inhibitory mechanisms of P fertilization on spore germination, on the growth and development of mycorrhizal hyphae, and on root mycorrhizal colonization have been observed in pure cultures (*Hepper*, 1983) and in soils (*Treseder* and *Allen*, 2002; *Jakobsen* et al., 2005). AMF abundance decreases with increasing nutrient availability across natural gradients of mean annual rainfall (*Bohrer* et al., 2001) and along with successional and environmental gradients (*Tao* and *Zhiwei*, 2005; *Zangaro* et al., 2014). As the main aim of the present research was to elucidate the temporal root trait plasticity and their interactions with typical soil AMF, we did not differentiate among various mycorrhizal guilds, neither had we included a separate treatment lacking soil AMF. The present study is a step forward because it demonstrates a gradual increase in root colonization by AMF in the successive plant growth stages. Moreover, this increase occurred without P fertilization, indicating that when P becomes limited, the symbiotic association of plant roots with AMF becomes increasingly important for plant P acquisition. The hairless mutant showed higher root colonization by AMF than WT maize (possessing root hairs). Although we did not identify AMF abundance in soil to correlate with root AMF colonization in the present study, a very recent study (*Boilard* et al., 2019), using almost the similar experimental setup with barley genotypes (with and without root hairs and with and without P fertilization), showed 3 times higher AMF abundance in the absence of root hairs and under low P availability. Thus, in the absence of root hairs (a key morphological trait for nutrient and water uptake), the mycorrhiza counteracts plant P acquisition. Such an increase in mycorrhizal colonization in the absence of root hairs demonstrates the importance of fungal partner. *Jakobsen* et al. (2005) reported higher root mycorrhizal colonization under nutrient limitation in *brb* (root hairless mutant) than in its wild type (with root hairs) in *Hordeum vulgare* cv. Pallas. The potential PHO activity was up-regulated with plant growth and was higher in unfertilized than P fertilized soils for both hairless and WT genotypes, highlighting higher P demand. Higher PHO activity is most likely due to increased AMF [significant correlation between AMF colonization and PHO activity (p = < 0.001); data not presented] and the corresponding symbiotic association with P-solubilizing bacteria producing PHO enzymes (*Zhang* et al., 2016). Such synergistic effects

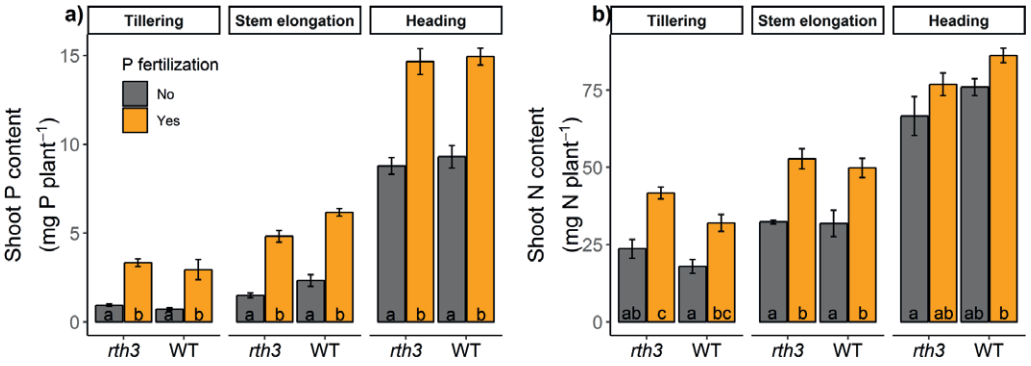

Figure 4: (a) Shoot P uptake (mg P plant^{-1} ± SE) and b) shoot N uptake (mg N plant^{-1} ± SE) of maize plants with (wild type: WT) and without root hairs (mutant: *rth3*). Letters indicate significant differences (two factor ANOVA, Tukey's post-hoc, $p < 0.05$) between genotypes and P fertilization at each growth stage: tillering, stem elongation, and heading.

of mycorrhiza and bacteria inoculation on plant growth have been demonstrated by *Boldt-Burisch* et al. (2018). With P fertilization, plant biomass and the P uptake in plants were higher from the onset, *i.e*., maize accumulates more P and invests it mainly in shoots, where it is stored (for example, in the storage lipids). At the maize heading stage, the lower P demand (the plants have already become established and switch their resource allocation to aboveground tissues) probably explains the suppression of PHO activity after P fertilization compared to unfertilized plants.

4.3 Plasticity of root traits

The present study empirically demonstrates that, in the absence of root hairs (*rth3* mutant), the average diameter of fine roots (AFRD) increases to facilitate AMF colonization. This increase in *rth3* maize with growth stage, along with the significant correlation ($p = 0.005$) between AFRD and mycorrhiza, highlights the requirement for more root volume to facilitate increased colonization. Several potential benefits for hairless maize are conceivable. An increased AFRD will: (1) provide more root volume to be colonized by AMF (*Reinhardt* and *Miller*, 1990), (2) increase root longevity (*Eissenstat*, 1992; *Comas* et al., 2014), which is beneficial to maintain the active exchange of nutrients and C between AMF and roots, (3) increase the root surface area for a given unit of root length in *rth3 versus* WT maize (*Haling* et al., 2013), and (4) reduce the metabolic costs such as root respiration in *rth3* maize (*Lynch* and *Ho*, 2005). Very recently, *Wen* et al. (2019) showed that PHO activity and carboxylates amount increased with increasing root diameter under soil P limitation. Such plastic responses of roots (increased AFRD and AMF colonization) are complementary to increase plant P acquisition, which is also a case in the present study. A strong contribution of AMF to shoot P uptake highlighted their importance in plant P acquisition under P limitation. The hairless mutant exhibited higher root colonization by AMF, emphasizing facilitation in the absence of root hairs, whereas the plants were less dependent on symbiotic fungi (AMF) for nutrient acquisition in the presence of root hairs. These results highlight that under P scarcity, root trait plasticity for nutrient acquisition is a common phenomenon.

5 Conclusions

Functional root traits and their plasticity are crucial for plant P uptake in P-limited soils. The absence of root hairs induced an increase in the average fine root diameter thereby promoting root mycorrhizal colonization by AMF (Fig. 5). In turn, the presence of root hairs decreased the dependency of plants on root mycorrhizal colonization for plant P acquisition. Reduced colonization after P fertilization highlighted the

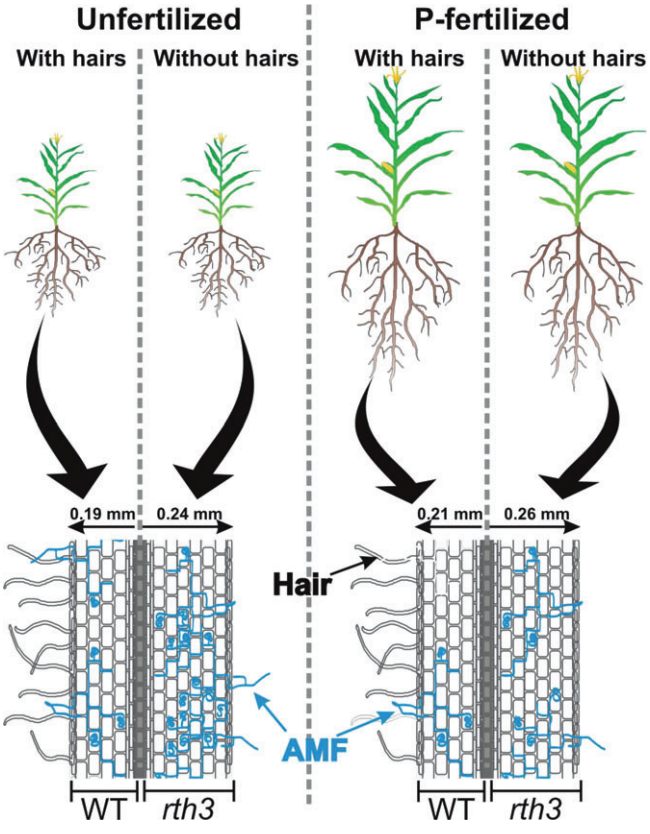

Figure 5: Illustration showing contrasting root traits in the presence (WT) and absence (*rth3*) of root hairs. When root hairs are absent, maize plants increased the average fine root diameter to facilitate root colonization by arbuscular mycorrhizal fungi to maintain P acquisition and growth.

maize plant's resource allocation by lowering trade-offs for P acquisition. Overall, maize alters its root morphology and biological traits as a nutrient acquisition strategy to maximize benefits and therefore, growth.

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References

Bakhshandeh, S., *Corneo, P. E.*, *Mariotte, P.*, *Kertesz, M. A.*, *Dijkstra, F. A.* (2017): Effect of crop rotation on mycorrhizal colonization and wheat yield under different fertilizer treatments. *Agr. Ecosyst. Environ.* 247, 130–136.

Bohrer, G., *Kagan-Zur, V.*, *Roth-Bejerano, N.*, *Ward, D.* (2001): Effects of environmental variables on vesicular-arbuscular mycorrhizal abundance in wild populations of *Vangueria infausta*. *J. Veg. Sci.* 12, 279–288.

Boilard, G., *Bradley, R. L.*, *Paterson, E.*, *Sim, A.*, *Brown, L. K.*, *George, T. S.*, *Bainard, L.*, *Carubba, A.* (2019): Interaction between root hairs and soil phosphorus on rhizosphere priming of soil organic matter. *Soil Biol. Biochem.* 135, 264–266.

Boldt-Burisch, K., *Naeth, M. A.*, *Schneider, U.*, *Schneider, B.*, *Hüttl, R. F.* (2018): Plant growth and arbuscular mycorrhizae development in oil sands processing by-products. *Sci. Total Environ.* 15, 30–39.

Brundrett, M. C. (2002): Biogeography of Mycorrhizal Symbiosis. Springer, Cham, Switzerland.

Brundrett, M., *Tedersoo, L.* (2019): Misdiagnosis of mycorrhizas and inappropriate recycling of data can lead to false conclusions. *New Phytol.* 221, 18–24.

Bukvić, G., *Antunović, M.*, *Popović, S.*, *Rastija, M.* (2003): Effect of P and Zn fertilisation on biomass yield and its uptake by maize lines (*Zea mays* L.). *Plant Soil Environ.* 49, 505–510.

Camenzind, T., *Homeier, J.*, *Dietrich, K.*, *Hempel, S.*, *Hertel, D.*, *Krohn, A.*, *Leuschner, C.*, *Oelmann, Y.*, *Olsson, P. A.*, *Suarez, J. P.*, *Rillig, M.* (2016): Opposing effects of nitrogen *versus* phosphorus additions on mycorrhizal fungal abundance along an elevational gradient in tropical montane forests. *Soil Biol. Biochem.* 94, 37–47.

Carbonnel, S., *Gutjahr, C.* (2014): Control of arbuscular mycorrhiza development by nutrient signals. *Front Plant Sci.* 5. DOI: https://doi.org/10.3389/fpls.2014.00462.

Chaparro, J. M., *Badri, D. V.*, *Vivanco, J. M.* (2014): Rhizosphere microbiome assemblage is affected by plant development. *ISME J.* 8, 790–803.

Chen, W., *Koide, R. T.*, *Adams, T. S.*, *DeForest, J. L.*, *Cheng, L.*, *Eissenstat, D. M.* (2016): Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. *Proc. Natl. Acad. Sci. USA.* 113, 8741–8746.

Comas, L. H., *Callahan, H. S.*, *Midford, P. .E.* (2014): Patterns in root traits of woody species hosting arbuscular and ectomycorrhizas: implications for the evolution of belowground strategies. *Ecol. Evol.* 4, 2979–2990.

Davidson, E. A., *De Carvalho, C. J. R.*, *Vieira, I. C. G.*, *Figueiredo, R. O.*, *Moutinho, P.*, *Ishida, F. Y.*, *Santos, M. T. R.*, *Guerrero, J. B.*, *Kalif, K.*, *Saba, R. T.* (2004): Nitrogen and phosphorus limitation of biomass growth in a tropical secondary forest. *Ecol. Appl.* 14, 150–163.

Eissenstat, D. M. (1992): Costs and benefits of constructing roots of smaller diameter. *J. Plant Nutr.* 15, 763–782.

Gahoonia, T. S., *Nielsen, N. E.*, *Lyshede, O. B.* (1999): Phosphorus (P) acquisition of cereal cultivars in the field at three levels of P fertilization. *Plant Soil* 211, 269–281.

Graham, J. H., *Eissenstat, D. M.* (1998): Field evidence for the carbon cost of citrus mycorrhizas. *New Phytol.* 140, 103–110.

Giovannetti, M., *Mosse, B.* (1980): An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.* 84, 489–500.

Haines, S. A., *Gehl, R. J.*, *Havlin, J. L.*, *Ranney, T. G.* (2015): Nitrogen and phosphorus fertilizer effects on establishment of giant Miscanthus. *Bioenerg. Res.* 8, 17–27.

Haling, R. E., *Brown, L. K.*, *Bengough, A. G.*, *Young, I. M.*, *Hallett, P. D.*, *White, P. J.*, *George, T. S.* (2013): Root hairs improve root penetration, root-soil contact, and phosphorus acquisition in soils of different strength. *J. Exp. Bot.* 64, 3711–3721.

Hepper, C. M. (1983): Effect of phosphate on germination and growth of vesicular-arbuscular mycorrhizai fungi. *T. Brit. Mycol. Soc.* 80, 487–490.

Hochholdinger, F., *Wen, T. J.*, *Zimmermann, R.*, *Chimot-Marolle, P.*, *Da Costa e Silva, O.*, *Bruce, W.*, *Lamkey, K. R.*, *Wienand, U.*, *Schnable, P. S.* (2008): The maize (*Zea mays* L.) *roothairless3* gene encodes a putative GPI-anchored, monocot-specific, COBRA-like protein that significantly affect grain yield. *Plant J.* 54, 888–898.

Holz, M., *Zarebanadkouki, M.*, *Kuzyakov, Y.*, *Pausch, J.*, *Carminati, A.* (2018): Root hairs increase rhizosphere extension and carbon input to soil. *Ann. Bot.* 121, 61–69.

Hooker, J. E., *Atkinson, D.* (1996): Arbuscular mycorrhizal fungi-induced alteration to tree-root architecture and longevity. *Z. Pflanz. Bodenkunde* 159, 229–234.

Jakobsen, I., *Chen, B.*, *Munkvold, L.*, *Lundsgaard, T.*, *Zhu, Y.-G.* (2005): Contrasting phosphate acquisition of mycorrhizal fungi with that of root hairs using the root hairless barley mutant. *Plant Cell Environ.* 28, 928–938.

Jungk, A. (2001): Root hairs and the acquisition of plant nutrients from soil. *J. Plant Nutr. Soil Sci.* 164, 121–129.

Kassambara, A. (2018): ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.1.7. Available at: https://CRAN.R-project.org/package=ggpubr.

Khalvati, M. A., *Hu, Y.*, *Mozafar, A.*, *Schmidhalter, U.* (2005): Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. *Plant Biol.* 7, 706–712.

Kiers, E. T., *West, S. A.*, *Denison, R. F.* (2002): Mediating mutualisms: farm management practices and evolutionary changes in symbiont co-operation. *J. Appl. Ecol.* 39, 745–754.

Kumar, A., *Shahbaz, M.*, *Blagodatskaya, E.*, *Kuzyakov, Y.*, *Pausch, J.* (2018): Maize phenology alters the distribution of enzyme activities in soil: Field estimates. *Appl. Soil Ecol.* 125, 233–239.

Kuzyakov, Y., *Xu, X.* (2013): Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytol.* 198, 656–669.

Lambers, H., *Shane, M. W.*, *Cramer, M. D.*, *Pearse, S. J.*, *Veneklaas, E. J.* (2006): Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. *Ann. Bot.* 98, 693–713.

Lambers, H., *Clements, J. C.*, *Nelson, M. N.* (2013): How a phosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (*Lupinus*, Fabaceae). *Am. J. Bot.* 100, 263–288.

Lambers, H., *Finnegan, P. M.*, *Laliberté, E.*, *Pearse, S. J.*, *Ryan, M. H.*, *Shane, M. W.*, *Veneklaas, E. J.* (2011): Phosphorus nutrition of proteaceae in severely phosphorus-impoverished soils: are there lessons to be learned for future crops? *Plant Physiol.* 156, 1058–1066.

Lenth, R. V. (2016): Least-squares means: The R package lsmeans. *J. Stat. Softw.* 69, 1–33.

Li, T., *Lin, G.*, *Zhang, X.*, *Chen, Y.*, *Zhang, S.*, *Chen, B.* (2014): Relative importance of an arbuscular mycorrhizal fungus (*Rhizophagus intraradices*) and root hairs in plant drought tolerance. *Mycorrhiza* 24, 595–602.

Lynch, J. P., *Ho, M. D.* (2005): Rhizoeconomics: Carbon costs of phosphorus acquisition. *Plant Soil* 269, 45–56.

Marx, M. C., *Wood, M.*, *Jarvis, S. C.* (2001): A microplate fluorimetric assay for the stud of enzyme diversity in soils. *Soil Biol. Biochem.* 33, 1633–1640.

Munyanziza, E., *Kehri, H. K.*, *Bagyaraj, D. J.* (1997): Agricultural intensification, soil biodiversity and agro-ecosystem function in the tropics: the role of mycorrhiza in crops and trees. *Appl. Soil Ecol.* 6, 77–85.

Péret, B., *Clément, M.*, *Nussaume, L.*, *Desnos, T.* (2011): Root developmental adaptation to phosphate starvation: Better safe than sorry. *Trends Plant Sci.* 16, 442–450.

Philippot, L., *Raaijmakers, J. M.*, *Lemanceau, P.*, *van der Putten, W. H.* (2013): Going back to the roots: the microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.* 11, 789–799.

R Core Team (2018): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.

Reinhardt, D. R., *Miller, R. M.* (1990): Size classes of root diameter and mycorrhizal fungal colonization in two temperate grassland communities. *New Phytol.* 116, 129–136.

Richardson, A. E., *Lynch, J. P.*, *Ryan, P. R.*, *Delhaize, E.*, *Smith, F. A.*, *Smith, S. E.*, *Harvey, P. R.*, *Ryan, M. H.*, *Veneklass, E. J.*, *Lambers, H.*, *Oberson, A.*, *Culvenor, R. A.*, *Simpson, R. J.* (2011): Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant Soil* 349, 121–156.

Schofield, E. J., *Rowntree, J. K.*, *Paterson, E.*, *Brooker, R. W.* (2018): Temporal dynamism of resource capture: a missing factor in ecology?. *Trends Ecol. Evol.* 33, 277–286.

Smith, S. E., *Smith, F. A.* (1990): Structure and function of the interfaces in biotrophic symbioses as they relate to nutrient transport. *New Phytol.* 114, 1–38.

Sundqvist, M. K., *Liu, Z.*, *Giesler, R.*, *Wardle, D. A.* (2014): Plant and microbial responses to nitrogen and phosphorus addition across an elevational gradient in subarctic tundra. *Ecology* 95, 1819–1835.

Tao, L., *Zhiwei, Z.* (2005): Arbuscular mycorrhizas in a hot and arid ecosystem in southwest China. *Appl. Soil Ecol.* 29, 135–141.

Treseder, K. K., *Allen, M. F.* (2002): Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytol.* 155, 507–515.

Veresoglou, S. D., *Menexes, G.*, *Rillig, M. C.* (2012): Do arbuscular mycorrhizal fungi affect the allometric partition of host plant biomass to shoots and roots? A meta-analysis of studies from 1990 to 2010. *Mycorrhiza* 22, 227–235.

Vierheilig, H., *Coughlan, A. P.*, *Wyss, U.*, *Piché, Y.* (1998): Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. *Appl. Environ. Microbiol.* 64, 5004–5007.

Wen, Z., *Li, H.*, *Shen, Q.*, *Tang, X.*, *Xiong, C.*, *Li, H.*, *Pang, J.*, *Ryan, M. H.*, *Lambers, H.*, *Shen, J.* (2019): Tradeoffs among root morphology, exudation and mycorrhizal symbiosis for phosphorus-acquisition strategies of 16 crop species. *New Phytol.* DOI: https://doi.org/10.1111/nph.15833.

Wen, Z., *Li, H.*, *Shen, J.*, *Rengel, Z.* (2017): Maize responds to low shoot P concentration by altering root morphology rather than increasing root exudation. *Plant Soil* 416, 377–389.

Wickham, H. (2016): ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York, NY, USA.

Zangaro, W., *de Almeida Alves, R.*, *de Souza, P. B.*, *Rostirola, L. V.*, *Lescano, L. E. A. M.*, *Rondina, A. B. L.*, *Nogueira, M. A.* (2014): Succession and environmental variation influence soil exploration potential by fine roots and mycorrhizal fungi in an Atlantic ecosystem in southern Brazil. *J. Trop. Ecol.* 30, 237–248.

Zhang, L., *Xu, M.*, *Liu, Y.*, *Zhang, F.*, *Hodge, A.*, *Feng, G.* (2016): Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. *New Phytol.* 210, 1022–1032.

Zhang, C., *Simpson, R. J.*, *Kim, C. M.*, *Warthmann, N.*, *Delhaize, E.*, *Dolan, L.*, *Byrne, M. E.*, *Wu, Y.*, *Ryan, P. R.* (2018): Do longer root hairs improve phosphorus uptake? Testing the hypothesis with transgenic *Brachypodium distachyon* lines overexpressing endogenous RSL genes. *New Phytol.* 217, 1654–1666.

