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What root traits determine grass resistance to phosphorus deficiency in production grassland?

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Abstract

Grasslands are a major form of agricultural land use worldwide. Current and future declines of phosphorus (P) inputs into production grasslands necessitate a shift towards selecting grass species based on high efficiency under suboptimal, rather than optimal P conditions. It is therefore imperative to identify key root traits that determine P acquisition of grasses in soils with a low P status. In a 9-month greenhouse experiment, we grew eight common grass species and cultivars on a soil with a low P status and related root morphological traits to their performance under P-limiting conditions. We applied (P1) or withheld (P0) P fertilization while providing adequate amounts of all other nutrients. Omitting P fertilization greatly reduced yield and nutrient acquisition for the various grass species. Biomass production differed significantly ($P < 0.1\%$) among species and P fertilization treatments, varying from 17.1 to 72.1 g pot^{-1} in the P0 treatment and from 33.4 to 85.8 g pot^{-1} in the P1 treatment. Root traits were species-specific and unresponsive to P fertilization, but overall we observed a trade-off between root biomass and specific root length. Structural equation modeling identified total root length as key factor with respect to resistance to P deficiency, especially when roots explored the subsoil. Optimizing root length and subsoil exploration could be the key to maintaining high productivity of production grasslands with decreasing P availability. This is relevant for both plant breeding programs and for composing seed mixtures.

Key words: P acquisiton / root characteristics / root length / structural equation modeling / yield

1 Introduction

Phosphorus (P) is one of the main nutrients for plant growth. Due to its immobile nature in soil, a low P availability is frequently limiting growth of crops in agricultural systems (*Hinsinger*, 2001). In agriculture, grasslands are a major form of land use across the world, covering approximately 26% of the Earth's ice-free surface (*Foley* et al., 2011), and accounting for 34% of Europe's agricultural land (*Eurostat*, 2016). Worldwide, grassland systems vary widely in their P inputs, P balances and yield outputs (*Sattari* et al., 2016; *Simpson* et al., 2014). Generally, production grasslands in temperate areas such as Europe and parts of North America are intensively managed, receive high P inputs in the form of manure and/or mineral fertilizer, and have high yields. Over the past decades however, P inputs in European grasslands have been decreasing (*Sattari* et al., 2016). A possible further reduction of P inputs in the future (due to stricter environmental legislation, or increased fertilizer costs), may result in sub-optimal P conditions and pose a challenge to maintaining high crop yields.

Maintaining high crop yields under reduced P inputs requires improvement of the P use efficiency in these grassland systems. One way to achieve this is the use of plants that are efficient in acquiring P. Plants have various strategies to increase P acquisition. They may for instance increase the volume of soil that is explored by the roots by increasing root : shoot ratios, alter their root distribution, increase the specific root length (SRL; root length per unit of weight), or increase topsoil foraging (*Lynch*, 2007). Additionally, plants may form mycorrhizal symbioses, or secrete protons, organic anions, and phosphatases to acquire P (*Richardson* et al., 2009). Sometimes several of these strategies can be combined, although trade-offs are also often observed (*Eissenstat*, 1992).

Studies on the differences in P acquisition among various grass species and varieties have previously been published, but the large majority focused on natural or semi-natural grasslands in which nutrients are far more scarce (*e.g. Hill* et al., 2006; *Fujita* et al., 2010). Studies focusing on the acquisition of P by grass species used in intensively managed systems are limited. *Caradus*, (1980) screened biomass production of a range of grass and legume species under two different P conditions (one of which was growth-limiting), but the study was carried out with small amounts of soil, making it difficult to translate these results to grassland systems. *Liu* et al. (1995) found significant differences in P acquisition and shoot P concentrations among various cultivars of *Poa pratensis* (Kentucky bluegrass), *Lolium perenne* (perennial ryegrass) and *Festuca arundinacea* (tall fescue) under moderate P fertilization. They showed that on average *F. arundinacea* had lower shoot P concentration than *P. pratensis* and *L. perenne*, but its total P acquisition was higher due to a higher yield. *Hamel* and *Heckman* (2006) found in their study that *P. pratensis* was more sensitive to P deficiency than *F. arundinacea* and *L. perenne*, and *Paredes* et al. (2011) reported a lower sensitivity of *F. arundinacea* to P deficiency compared to *L. perenne*. These studies indicate that differences exist among grass species in P acquisition, but it remains unclear which plant traits are underlying these differences.

Variation of root traits among grass species may be driving these differences in plant P acquisition. For example, *Elberse* and *Berendse* (1993) reported variation among eight grass species in responsiveness to nutrient availability. They found that species from nutrient-poor environments such as *Anthoxanthum odoratum* and *Festuca rubra* tend to have a higher SRL compared to those from nutrient-richer environments such as *L. perenne* and *Arrhenatherum elatius*. This may indicate that species with higher SRL are better accustomed to retrieving P

in a P-limiting environment. Many studies have reported differences in rooting depth, SRL and other root morphological traits among grass species and cultivars (*e.g. Crush* et al., 2007; *Deru* et al., 2014). These characteristics may change with the presence or absence of P fertilization (*Hill* et al., 2006). Also, grass species may be affected differently by the presence of arbuscular mycorrhizal fungi (*Unger* et al., 2016; *Wilson* and *Hartnett*, 1998) or may secrete different quantities of phosphatases at low P availability (*Olde Venterink* and *Güsewell*, 2010).

Due to crop selection under well-fertilized conditions, grass species and varieties used in production grasslands have not been selected based on their root traits. They are selected based on yield potential under optimal nutrient conditions (*Whitehead*, 2000; *Smit* et al., 2005), rather than their root characteristics. With a further movement towards reduced P inputs, and a possible decrease of the soil P status, yield of grass species under suboptimal conditions will become increasingly important. This may lead to a greater role for species and genotypic differences in root traits and P acquisition strategies among plants as seed selection criteria for farmers and plant breeders.

In the current study, we aim to link the performance of commonly seeded grass species, in the absence and presence of P fertilization, to root morphological traits. We hypothesize that, when comparing P-limited and P-sufficient conditions experimentally, (i) species with a relatively high yield and P uptake under P-sufficient conditions, will be affected to a greater extent when P is withheld and (ii) differences in root biomass distribution and SRL are the main drivers for the differences in performance among species in the absence of P fertilization. We tested these hypotheses in a nine-month pot experiment where grass species

were grown on a low-P non-calcareous sandy soil, and P fertilization was either applied or withheld.

2 Materials and Methods

2.1 Soil collection and physico-chemical characteristics

In our greenhouse experiment, we used the same soil as in a previous study (*Vos* et al., 2014). The soil (Umbric Gleysol; *FAO,* 2006) was collected from the 0-20 cm soil layer of a pasture near the village of Joppe, the Netherlands (52°20'N, 6°23'E) in April 2013. Prior to the experiment, the soil was air-dried and sieved to pass 5 mm. Physico-chemical soil properties (Tab. 1) were taken from *Vos* et al. (2014). Before determining these properties, the soil was oven-dried (40°C) and sieved (2 mm). In short, particle size distribution was determined using the pipette method and organic matter (OM) content was estimated from loss-on-ignition (*Houba* et al., 1997). In this study, water-extractable dissolved inorganic phosphorus (DIP) was measured as described in *Vos* et al. (2014): A soil sample was extracted at a solid to solution ratio of 1 : 10 (w : v) for 24 h at 75 strokes min^{-1}, centrifuged for 15 min at 10 000 rpm, and filtered (0.45 µm; Aqua 30/0.45 CA Whatman). The DIP concentration was subsequently determined using the molybdenum blue method (*Murphy* and *Riley,* 1962) and segmented flow analysis (SFA; Skalar, SAN^{++}). Before centrifugation and filtration of the same water extracts, the pH_{H2O} was measured in a subsample of the suspension. Organic soil P was calculated as the difference between the amounts of P extracted with 0.5 M H_2SO_4 from an ignited soil sample (550°C; total soil P) and an unignited sample (inorganic soil P; *Kuo,* 1996). The pool of P reversibly bound to reactive metal (hydr)oxides (P_{ox}) was determined using the acid ammonium oxalate extraction method (*Schwertmann,* 1964). Concentrations of P, Al (Al_{ox}), and Fe (Fe_{ox}) in the acid ammonium oxalate extracts were measured using

inductively coupled plasma – atomic emission spectroscopy (ICP-AES; Varian Vista Pro). From these results, the P saturation index (α) was calculated:

$$\alpha = \frac{P_{ox}}{(Fe + Al)_{ox}} \quad (1)$$

with P_{ox} and $[Fe + Al]_{ox}$ in mmol kg^{-1} (*Van der Zee* and *Van Riemsdijk*, 1988). Ammonium lactate-extractable P (P-AL) was used to determine the agronomic P status of our soil for grassland (*Egnér* et al., 1960).

2.2 Experimental design

We examined the growth and acquisition of eight commonly used grass species/cultivars in the presence and absence of P fertilization in a 9-month greenhouse experiment. The experiment was set up as a full-factorial, completely randomized block design, with two P fertilization treatments (P fertilization absent or present: P0 and P1) aimed at either creating P-limiting or optimal growth conditions, and eight grass species/cultivars (hereafter referred to as 'grass species') as the two independent factors. Each treatment was replicated six times, resulting in a total of 96 pots at the start of the experiment. The replicates were evenly distributed over three blocks, resulting in two replicates per block, one of which was destructively harvested after 118 d and the other after 275 d. Conditions in the greenhouse were semi-controlled: Temperature was kept above 15°C at all times and during winter a 16 h day was maintained by using artificial light as a supplement to natural light. Additionally, relative air humidity was kept at 65%.

For the experiment, the 96 polyvinylchloride pots (15 cm in diameter and 40 cm in height) were filled with 8 kg air-dried, sieved (5 mm), and homogenized soil, mixed with fertilizer solution, and water. All pots were fertilized with nitrogen (N; 27 mg kg^{-1}, supplied in equal molar amounts of ammonium and nitrate), potassium (K; 24 mg kg^{-1}), calcium (Ca; 11 mg kg^{-1}), sulfur (S; 3.3 mg kg^{-1}), and sodium (Na; 2.4 mg kg^{-1}). The pots of the P1 treatment received additional P (9.7 mg kg^{-1}, in the form of K_2HPO_4). The composition of the nutrient solution can be found in Tab. 2. The water (up to a total of 1130 mL per pot) was added to bring the soil moisture up to 60% of the maximum water-holding capacity (WHC). On top of this mixture, an unfertilized germination layer was added using 250 g of dry soil and 36 mL water.

At the start of the experiment, per pot 1 g of grass seeds of one of the following eight species was sown in the germination layer of the pots: a diploid cultivar of *L. perenne* (LP2; cv. Barforma), a tetraploid cultivar of *L. perenne* (LP4; cv. Bealey), *Lolium multiflorum* (LM; annual ryegrass; cv. Barelli), *F. arundinacea* (FA; cv. Bardoux), *Poa trivialis* (PT; rough bluegrass; cv. Bartalon), *Poa pratensis* (PP; cv. Bariris), *Phleum pratense* (PHP; Timothy-grass; cv. Barpenta), and *Holcus lanatus* (HL; velvet grass; cv. Barlatus). Seeds from all these grasses were obtained from Barenbrug Holland B.V., Nijmegen, the Netherlands. For the first 3 d, pots were covered with plastic to keep the soil moist and stimulate seed germination. Soil moisture was kept at 60% of maximum WHC throughout the rest of the experiment. This was done by gravimetrically checking moisture loss in five to six randomly selected pots, and watering accordingly with tap water once every 2 d. Once per week, all pots were weighed individually and both the setup of the pots within the blocks and the location of the blocks were randomized.

During the experiment, eleven grass cuts were collected, with 25 ± 4 d between each cut. In every pot, grass was cut at 4 cm above the soil surface. These cuttings were then dried at 70°C for 24 h and weighed. The material was milled (1 mm) and N and P concentrations (mg g^{-1}) in the grass were determined using a digestion with H_2SO_4, salicylic acid, H_2O_2, and selenium as a catalyst (*Temminghoff* and *Houba*, 2004). Shoot N and P uptake (mg pot^{-1}) were subsequently derived per cut from yield and shoot N and P content. After every cut, pots were fertilized again by broadcasting nutrient solution at the soil surface so that only P was limiting growth in the P0 treatment and so that optimal growth was achieved in the P1 treatment (see Tab. 1 for fertilizer rates and composition). As we suspected the initial fertilizer regime to be inadequate, fertilization rates were increased after the fifth cut to ensure these conditions were maintained.

2.3 Root and soil collection and analysis

After the fifth cut (118 d) and after the last and eleventh cut (275 d), the two halves of the experiment were destructively sampled and root samples were collected. More specifically, 3 pots from every treatment were sampled during the first sampling, with one pot from each block. The remaining three pots of each treatment were sampled during the second sampling. For the destructive sampling, the pots were removed from the experiment after cutting the grass, and the topsoil (0-10 cm) was separated from the subsoil (10-40 cm) using a knife to enable collection of roots from both soil layers. Roots were removed from the soil by hand over a sieve and subsequently rinsed with water until visually free of soil particles. At this point, root subsamples from the topsoil and subsoil were taken for root scanning and mycorrhizal colonization measurements and these samples (approximately the equivalent of

0.05-0.10 g dry weight) were stored in 50% ethanol until further analysis (*Pérez-Harguindeguy* et al., 2013). The remainder of the roots was dried at 70°C for 24 h and weighed. After the roots had been taken out, the remaining soil was mixed and samples were taken from both topsoil and subsoil. These samples were dried at 40°C and stored.

For the topsoil samples from the pots in one block taken after the eleventh cut, a water extraction (1 : 10, w : v) was used, following the procedure of *Vos* et al. (2014) as described above, to obtain an indication of the amount of readily available P at the end of the experiment. In these water extracts, we measured total dissolved P (TDP) using ICP-AES, and DIP, total dissolved N (TDN), nitrate-N ($N\text{-}NO_3$), and ammonium-N ($N\text{-}NH_4$) using SFA. Dissolved organic N (DON) was calculated as the remaining pool when subtracting $N\text{-}NO_3$ and $N\text{-}NH_4$ from TDN. Additionally, we measured the pH in the water extracts.

To assess the degree of mycorrhizal colonization, the root samples were removed from the ethanol, rinsed, and cut into 1 cm segments. These segments were washed and stained with Trypan Blue dye (*Phillips* and *Hayman*, 1970). The degree of root colonization was assessed microscopically (*Giovannetti* and *Mosse*, 1980). For the root scanning, subsamples were dyed with 0.5 g L^{-1} Natural Red dye for 24 h (*Bouma* et al., 2000). Images were acquired using an Epson Perfection V700 Photo scanner at 600 dpi and analyzed for root length and root diameter using the WinRhizo Pro 2013e software (Regent Instruments Inc., Quebec City, QC, Canada). After scanning, the root subsamples used were dried at 70°C for 24 h and weighed to calculate SRL. The root mass of these subsamples was added to the biomass of the remainder of the roots to calculate total root biomass and shoot : root ratio. From this, subsoil

exploration was calculated as the root biomass found in the subsoil (10-40 cm) divided by the total root biomass.

2.4 Data analyses

We used two-way analysis of variance (ANOVA) in GenStat 17th edition (VSN International Ltd, Hemel Hempstead, UK) to analyze whether there were significant effects of the factors P treatment and grass species on different response variables ($P < 5\%$). If necessary, data were transformed prior to analysis to conform to assumptions on normality and homogeneity of variances. We used power transformations (shoot yield, N acquisition, shoot N : P ratio, root mass in both top- and subsoil, SRL of topsoil roots, root length in top- and subsoil), log transformations (shoot : root ratio, SRL of subsoil roots) and double log transformations (relative subsoil exploration, mycorrhizal colonization in subsoil roots). As a post-hoc test, Tukey's honest significant difference test was applied to differentiate between treatments.

We assessed the correlation between different response variables and their importance for explaining the variation among species with redundancy analysis (RDA; *Rao*, 1964). The factor grass species was used as an environmental factor, whereas the shoot and root traits were the response variables. The significance of the canonical axes was tested using a Monte Carlo permutation test using 999 permutations. The analysis was done separately for both P treatments, and was performed in Canoco 5 for Windows.

Structural equation modeling (SEM) was applied to identify the most important pathways determining yield of the grass. This method is used to determine whether our proposed causal

relationships based on *a priori* knowledge match with the empirical results of our experiment (*Grace*, 2006). We used the Lavaan package (*Rosseel*, 2012) in R version 3.2.2 to execute the analysis. Values of the predicting and dependent variables used in the SEM were transformed beforehand to achieve comparability of parameters. We defined our conceptual model *a priori* based on knowledge from literature, and tested the quality of this model separately for the two P treatments. For the root characteristics in the model, we did not make a distinction between topsoil and subsoil. Hence, for root length and root mass, the values for topsoil and subsoil were added, and for SRL and mycorrhizal colonization we used a weighted average based on root mass in both soil layers. After the model was run, we used the modification index function to find omitted pathways that might improve the model. The quality of the conceptual models was concluded from the χ^2 statistic (with good model fits providing insignificant results, P > 5%), and any alterations made to the model were evaluated using the Akaike (AIC) and Bayesian (BIC) information criteria, which are mainly used to compare different models and penalize for model complexity. Within the tested models, pathways with a P value lower than 5% were assumed to be significant.

3 Results

3.1 Soil characteristics

Table 2 shows the physico-chemical characteristics of the soil used in the pot experiment. Based on the P-AL value of 26 mg P kg^{-1}, the P status of this soil in the Netherlands is considered low (*Tunney* et al., 1997). More than 50% of the total soil P content was organic P. The remainder of the total soil P content, i.e. inorganic P, was mostly adsorbed to metal (hydr)oxide surfaces, as indicated by the low α. In Table 3 the amounts of water-extractable (1 : 10, w : v) P and N species in the top soil of the pots at the end of the experiment are

given. In the soil samples from the treatment without P fertilization, DIP and TDP concentrations remained below detection limits. With P fertilization, DIP and TDP concentrations were detectable but still rather low (0.04-0.06 mg L^{-1} for DIP and 0.1-0.2 mg L^{-1} for TDP), even though they were slightly above the DIP concentration of 0.02 mg L^{-1} for the initial soil (Tab. 2). Concentrations of $N\text{-}NH_4$ and $N\text{-}NO_3$ at the end of the experiment ranged from 0.2-5.4 mg L^{-1} and 2.8-40.4 mg L^{-1} respectively (Tab. 3).

3.2 Yield and nutrient acquisition

Results after five cuts (118 d) showed almost identical, albeit slightly less pronounced patterns to those obtained after eleven cuts, for yield and nutrient uptake as well as root traits. We will therefore limit the discussion of the results to those obtained at the end of the experiment (275 d). After eleven cuts, cumulative yields ranged from 17.1 g pot^{-1} (PT, P0) to 85.8 g pot^{-1} (HL, P1) (Fig. 1a; Tab. 4). Biomass yield was significantly affected by P treatment as well as grass species, and there was no interaction between these factors (Tab. 4). Relative yield performance in the absence of P fertilization (the yield in the P0 treatment divided by the yield in the P1 treatment) differed significantly per species and was lowest for PT, whereas LM, FA, HL and PP showed the smallest relative yield decline when P fertilization was withheld (Fig. 1b). Similar results were observed for P and N uptake throughout the experiment (Tab. 4, Fig. 1c and 1d). Cumulative P uptake ranged from 30 mg pot^{-1} (PT, P0) to 218 mg pot^{-1} (HL, P1) (Fig. 1c; Tab. 4). As for yield, relative P uptake in the absence of P fertilization (the P uptake in the P0 treatment divided by the P uptake in the P1 treatment) was lowest for PT, differing significantly from FA and LM, which had a high relative P uptake. The average N : P ratio in the shoots harvested throughout the experiment was the only response variable that showed an interaction between P fertilization and grass species (Tab. 4). They varied from 19.8 (HL) to 33.4 (PT) in the P0 treatment and from 14.5

(LM) to 22.1 (PT) in the P1 treatment. All species had their N : P ratio reduced as a result of P fertilization, but PT in particular was more affected than other grass species. Overall, the highest N : P ratios were found for PT, while LM and HL had the lowest N/P ratios.

3.3 Root traits

Table 5 shows the different root parameters per P treatment and grass species. All root traits differed significantly among grass species in both the topsoil and subsoil layers, except for SRL in the topsoil, whereas the P treatment generally did not significantly affect the root traits. Overall, the largest root mass was observed for LM, whereas PP had the smallest biomass (Table 5), and species with large root mass also had a high shoot yield (Fig. 1a). The relative subsoil exploration also differed per species: FA and HL had a relatively high fraction of their root mass in the subsoil (10-40 cm), whereas for PT almost all roots were allocated in the topsoil. For SRL we found high values (up to 1280 m g^{-1}) for PT, PP, and PHP, while FA and LM had low SRL (Tab. 5). Additionally, a trade-off was observed between root mass and SRL in both P treatments, with SRL declining as root mass increased (Fig. 2).

The degree of mycorrhizal colonization was the only root trait that was significantly affected by P treatment (Tab. 5). In both P treatments, the average mycorrhizal colonization in grass roots ranged from 8% to 74% in the topsoil and 11% to 77% in the subsoil; P fertilization decreased colonization. Mycorrhizal colonization and SRL in the subsoil were affected by the interaction of P treatment and grass species.

3.4 Redundancy analysis and structural equation modeling

Results of the RDA are shown in Fig. 3. The canonical axes were significant for both P0 (P = 0.2%) and P1 (P = 0.2%) treatments, and the first two canonical axes cumulatively explained 70% and 66% of the variation for P0 and P1, respectively. Plant yield and nutrient uptake were highly positively correlated with root mass, root length and relative subsoil exploration, and negatively related to SRL, shoot N content and N : P ratio. Mycorrhizal colonization showed no or little correlation with the aforementioned plant traits. In both P treatments, LM was correlated with variables such as root mass and root length, whereas PHP and PP were mostly related to SRL and shoot : root ratio.

In the SEM analysis, the conceptual model defined *a priori* (Fig. 4c) was not significant (P > 5%) for either P treatment. However, based on the RDA results (Fig. 3), the relationship between mycorrhizal colonization of roots and yield was omitted from the conceptual model. Additionally, the modification index function suggested the inclusion of a pathway between SRL and shoot yield. The resulting final model matched the data well and performed the best in explaining the data for both the P0 (model fit χ^2 statistic P = 37%, RMSEA = 0.05; Fig. 4a) and the P1 (P = 35%, RMSEA = 0.07; Fig. 4b) treatments. The strongest relationships were found between root mass and root length, as well as root length and yield, indicated by the standardized relationship coefficients (Fig. 4a and 4b). Besides total root length and root mass, relative subsoil exploration and SRL also directly influenced shoot yield. Positive relationships between mycorrhizal colonization and root mass, and between subsoil exploration and root mass, were found in the presence of P fertilization, but not in its absence. There was no significant relationship between SRL and mycorrhizal colonization for either P treatment.

4 Discussion

4.1 Yield and nutrient uptake

Grass yield and nutrient uptake over the entire experiment were significantly lower when P fertilization was omitted. All eight grasses showed decreases for shoot yield, P uptake and N uptake in the P0 treatment as compared to the P1 treatment (Tab. 4; Fig. 1). Although in general these effects were highly significant and omitting P fertilization resulted in large reductions of yield and P uptake (with decreases ranging from 13% to 50% for yield and from 27% to 57% for P uptake), differences between P0 and P1 within individual species were often not significant. Only LP2 showed a significant yield increase when fertilized with P (Fig. 1a). The influence of P fertilization was more pronounced with respect to P uptake, for which we found a significant increase for five out of eight grass species (Fig. 1c and 1d). This is to be expected, as plants may respond to P deficiency with lower P concentration in shoots and a reduction in shoot development (*Ha* and *Tran*, 2014; *Richardson* et al., 2009) and P uptake is a combined effect of these two responses.

We hypothesized that species with a relatively high yield and P uptake under P-sufficient conditions would be affected to a greater extent when P is withheld. However, the lack of interactions between grass species and P treatment for yield and nutrient uptake (Tab. 4) indicates that withholding P fertilization resulted in similar decreases (in absolute sense) in both yield and P uptake. Moreover, the species that perform best in the P0 treatment in terms of biomass production (HL and LM) also tended to have high yield under P fertilization, and both these species lost relatively little biomass when P fertilization was withheld (Fig. 1b). Grass species that had a lower yield and nutrient uptake were relatively more affected by the lack of P fertilization. The only exception to this pattern was PP, which had low yield and

nutrient uptake compared to other grass species, but showed only small decrease when P fertilization was withheld. Overall however, higher-yielding species were more resistant to a reduction in P availability. Contrary to our expectations, our first hypothesis is therefore rejected by our data. Possible reasons for this will be discussed below.

4.2 Root traits

Root traits in our experiment varied significantly among species, but were not affected by P treatment (Tab. 5). Only mycorrhizal colonization was significantly decreased in the presence of P fertilization; this is in line with earlier observations (*Hill* et al., 2010; *Mendoza* et al., 2016). Despite pronounced differences for root mass and SRL, total root length in the top 10 cm of the soil was the one trait that did not significantly differ among grass species. This is likely due to the dense rooting in the top soil of grass species in general, a common feature that might have been further stimulated in this experiment by applying nutrients on the soil surface after every cut. Overall, a finer resolution of the root distribution analysis across the soil profile (for instance by taking an additional sample from the top few cm), might have been able to capture this effect and to show clearer differences among species. The large root mass found for LM in both P treatments indicates a fast growing species that roots very thoroughly. This is in agreement with results found earlier (*Crush* et al., 2005; *Moir* et al., 2013). Many of the higher-yielding species that performed relatively well both in presence and absence of P fertilization (LM, FA, and HL) had a relatively large part of their roots in the subsoil (10-40 cm). In contrast, the two *Poa* species had the largest SRL of all species, but had lower root biomass and yield. The differences in root traits among the grass species point towards different below-ground strategies to increase the amount of explored soil and nutrient uptake by increasing SRL, root mass and/or subsoil exploration. The lack of effect of P fertilization on the measured root traits suggests that the plasticity of these traits (i.e. the

capacity to adapt to changes in P availability) in the grass species used in our pot experiment is limited. The ability of these species to acquire P in P-limiting environments would therefore be based on intrinsic root characteristics, rather than the phenotypic plasticity of these characteristics (*Fransen* et al., 1999; *Hill* et al., 2006). *Freschet* et al. (2015) did find phenotypic plasticity in herbaceous species as a response to nutrient treatments, but these treatments did not include P limitation. Alternatively, the grasses in our experiment may have responded by changing root characteristics we did not measure such as the speed of root system development or rooting depth. The different grass species may furthermore have increased the production of organic compounds or phosphatases (*Olde Venterink* and *Güsewell*, 2010; *Richardson* et al., 2009), which would have resulted in root physiological plasticity, rather than root structural plasticity.

Another factor that is likely to have contributed to the absence of a P effect on the different root traits is the soil, which was very low in readily available P (Tab. 1). Despite our efforts to create a pronounced difference in readily available P by using presence/absence of P fertilization, other soil characteristics might have reduced this effect. The amounts of water-extractable P found in the topsoil at the end of the experiment indicate that P fertilization only slightly increased readily available P in the soil during our experiment (Tab. 1; Tab. 3). This increase was barely detectable by our measurements and consisted mostly of organic or unreactive P (TDP-DIP). The large amounts of Fe_{ox} and Al_{ox} in our soil indicate a high content of amorphous metal (hydr)oxides (*Koopmans* et al., 2006). This leads to a low P loading of the metal (hydr)oxides as evidenced by the low α value of 0.06 (Tab. 2). If we take into account all additions of fertilizer P supplied throughout the experiment, the average α would increase to 0.07. The maximum value of α varies between 0.4 and 0.6 (*Van der Zee* et al., 1988; *Van der Zee* and *Van Riemsdijk*, 1988), meaning that our soil still has the capacity

to adsorb additional P. A large part of the P that was introduced to the soil through P fertilizer application in the P1 treatment may therefore have been adsorbed by metal (hydr)oxides, resulting in P-limiting conditions despite the addition of P. More evidence for the mild P deficiency can be found in the N : P ratios of the shoots (Tab. 4). *Koerselman* and *Meuleman* (1996) found wet grasslands to be P-limited for N : P ratios above 16 and N-limited for ratios below 14. Slightly larger ranges (below 10 for N limitation and above 20 for P limitation) for N : P ratios have also been reported (*Güsewell*, 2004). The average values for N : P ratios in the P0 treatment are close to, or higher than 20 for all grass species, which indicates a substantial P deficiency. For the P1 treatment, most ratios were above 16 (all except LM and HL), suggesting a milder P deficiency. The combination of these data provides indirect evidence that grass growth in both the P0 and P1 treatment might have been P-limited. Overall, the difference in P availability between P0 and P1 treatments seemed to be large enough to cause differences in yield and nutrient uptake, but not to significantly affect root traits of the different grass species under the current experimental setup.

4.3 Interactions between root traits and yield

We observed a trade-off between SRL and root mass for both P treatments (Fig. 2). The grass species with higher yields and nutrient uptake balanced these two properties (HL) or had higher root mass (LM), whereas the lower-yielding species (PT, PP) prioritized SRL. This trade-off is also supported by the results of the RDA, which indicate that SRL correlates positively with shoot : root ratio and negatively with root mass (Fig. 3). Trade-offs like this have been reported before, not only between SRL and root mass (*Eissenstat*, 1992; *Freschet* et al., 2015), but also for example between SRL and mycorrhizal colonization (*Cortois* et al., 2016; *Smith* and *Read*, 2008). Our SEM analysis was aimed at testing whether these trade-offs were present and at finding the most influential pathway through which the grass species in

our experiment acquired P. To do so we included relationships between root mass, SRL and mycorrhizal colonization in our conceptual model, next to those between these root traits and yield (Fig. 4c). Many of the defined relationships between different root traits and yield in our conceptual model were significant (Fig. 4a and 4b). The relationships between root mass and root length, and root length and yield were strongly positive, as expected, and seemed to have the largest impact. Total root length over the entire soil profile appears to explain the variation in yield to a significant extent.

A priori we expected SRL to be an important discriminator among our grass species in their susceptibility to P deficiency. However, the SEM showed that the relation between SRL and yield in our system was complex. Next to the earlier observed trade-off between SRL and root mass, we found an indirect relation of SRL with yield through total root length, as well as a direct negative relationship between SRL and yield. The latter is an effect we did not include in our conceptual model, but was suggested by the modification index function. We do not have a clear explanation for this significant negative relation between SRL and yield. However, it does indicate that there are other, yet un-quantified, underlying traits associated or correlated with high SRL that constrain plant biomass production. These may be plant physiological properties, such as a trade-off between phosphatase activity and SRL. The expected trade-off between SRL and mycorrhizal colonization was not significant for either P treatment. Overall, the coefficients of the SEM show that SRL is negatively correlated with yield (which is further supported by the RDA; Fig. 3). Subsoil exploration appeared to affect yield positively, either directly (by exploring a larger volume of soil), and indirectly (by allowing for more total root biomass). However, total root length rather than SRL or subsoil exploration was the determining factor for yield, in both P treatments. This means our second hypothesis was not confirmed.

Although our model explains a large proportion of our data (R^2 values of 0.86 and 0.92 for P0 and P1 respectively), and performs well on the χ^2 and RMSEA statistics for both P treatments, there is still room for improvement. In particular, by adding other factors and strategies of plants that we did not take into account. Most prominently, we did not measure phosphatase activity or P mineralization rates from OM in this experiment. In a study on N and P stoichiometry in grasslands, *Fujita* et al. (2010) reported differences of a factor three in relative phosphatase activity among grass species. Moreover, root surface phosphatase activity was correlated with grass P uptake (*Fujita* et al., 2010). Root surface phosphatase activity may thus differ among various grass species, and could have contributed to grass P uptake in a soil with a relatively high OM content like ours (Tab. 1). Furthermore, root hairs might increase P acquisition by exploring a larger volume of soil. The length and density of these root hairs may differ for grass species, and are often affected by environmental conditions such as nutrient availability or acidity (*Haling* et al., 2011; *Robinson* and *Rorison*, 1987; *Yang* et al., 2015). These are examples of properties that could be included in further studies towards the importance of different root traits for P acquisition. Finally, the ratio between shoot and root biomass was not explicitly included in the model as a predictor. However, this effect is indirectly taken into account by including a negative relationship between root mass and yield (Fig. 4).

4.4 Implications

For natural or semi-natural grasslands, differences in P acquisition among various grass species and varieties have received considerable attention. However, this has not been the case for P acquisition by grass species in intensively managed production grasslands. Our

study shows that root traits such as root length and subsoil exploration can be important characteristics in determining yield and P uptake of grass species commonly grown in production grassland under P-limiting conditions, and thus their resistance to P deficiency. Translating results from pot experiments to field conditions is always a challenge, as growth rate and rooting characteristics of plants in the greenhouse generally differ from those in the field (*De Vries*, 1980). Additionally, the nutrient management in our experiment was aimed at creating P limitation or removing nutrient limitation. To ensure this, large quantities of inorganic nutrients were applied, which is not representative for nutrient management in grassland systems in practice, where application rates are lower and often include organic sources such as manures. Different root traits might be beneficial for acquiring P from organic sources. The fact that we used a mixed topsoil, instead of an intact soil core with a P stratification or nutrient gradient that usually occurs in the field (*Whitehead*, 2000), may also have affected nutrient distribution and the rooting pattern of the grasses. The results we obtained for subsoil exploration should be regarded with this in mind, as it is not certain that this trait will be as important in the field as it was in this pot experiment. Rather, the root development of grasses in the top few cm of the soil might be a key root trait, as this is where most of the P will end up after application.

Finally, the limited pot volume may have impaired root growth and with that limited root morphological plasticity over the duration of the experiment. However, the large depth of our pots provided a closer approximation to realistic rooting conditions than most pot experiments, as most often shallower pots are used. Additionally, the long duration of this experiment, in combination with the relatively stable climate in the greenhouse, provides unique conditions for studying fundamental relations between rooting patterns and P acquisition of grass that are not easily obtained in the field. The results of our experiment

suggest that selecting (combinations of) grass species based on root length and soil exploration might be a key to maintaining high grass yields and P uptake in production grasslands. However, this needs further confirmation in the field and on different soil types. Future research should also focus on grass species that have complementary root traits (for instance a combination of deep-rooting and shallow-rooting species). This might provide useful information for plant breeding programs or composing seed mixtures.

5 Conclusions

In this experiment, omitting P fertilization to grass species commonly occurring in intensively managed grasslands resulted in large reductions of both yield and nutrient acquisition. We showed that grass species differ in their resistance to P deficiency, with biomass production and nutrient acquisition differing significantly among species in the absence or presence of P fertilization. Root traits generally varied among species, but were not affected by P treatment, which indicates limited plasticity of these traits under the current experimental setup. We observed a trade-off between SRL and root biomass, but SEM showed that total root length and subsoil exploration were the root traits that explained most of the variation in our yield data. Selecting grass species based on these root traits for plant breeding programs or use in grass seed mixtures may be a key to maintaining optimal yields under declining soil P status, as a result of lower P inputs.

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711 **Table 1:** Physico-chemical characteristics of the soil.

Characteristics	Bulk soil
Sand (%)	67
Silt (%)	22
Clay (%)	11
Organic matter (g kg^{-1})[a]	85
pH_{H2O}	5.6
Water-extractable DIP (mg L^{-1})[b]	0.02
P-AL (mg P kg^{-1})	26
Total P (mg kg^{-1})	823
Organic P (mg kg^{-1})	439
Al_{ox} (mmol kg^{-1})	16.9
Fe_{ox} (mmol kg^{-1})	169.9
P_{ox} (mg kg^{-1})	334
α[c]	0.06

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713 [a]Loss on ignition with the loss of water from the crystalline structure of clay taken into account.

714 [b]Concentration of dissolved inorganic phosphorus (DIP) measured in a 1:10 (w:v) water extract (see Material

715 and Methods for details).

716 [c]Degree of P saturation of a soil with respect to its content of reactive metal oxides calculated according to

717 Equation 1.

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Table 2: Fertilizer application throughout the experiment. The elements were supplied using a mixture of the following salts: NH_4NO_3; NH_4Cl; $(NH_4)_2SO_4$; KNO_3; KCl; KH_2PO_4; $CaCl_2$; NaCl and $MgSO_4$. Fertilizer solutions for the P0 and P1 treatments had the same ionic strength and equal amounts of NH_4^+ and NO_3^-.

	Fertilizer applied (kg ha^{-1})						
Time in the experiment	N	P[a]	S	K	Ca	Na	Mg
Start of the experiment	120	44	15	108	50	11	
Cut 1 (25 days)	70	11	15	83		11	
Cut 2 (46 days)	70	11		83			
Cut 3 (74 days)	70	11		83			
Cut 4 (95 days)	70	11		83			
Cut 5 (118 days)	70	44		83			
Cut 6 (145 days)	70	44		83			
Cut 7 (170 days)	250	44	76	279	239		58
Cut 8 (194 days)	250	44	76	279	239		58
Cut 9 (221 days)	250	44	76	279	239		58
Cut 10 (248 days)	250	44	76	279	239		58

[a]Phosphorus was only fertilized to pots of the P1 treatment.

747 **Table 3:** Composition of the water extracts (1 : 10, w : v) performed on the top soil of the pots in block 1 after

748 11 cuts.

Grass species	P treatment[a]	TDP (mg L^{-1})	DIP (mg L^{-1})	$N\text{-}NH_4$ (mg L^{-1})	$N\text{-}NO_3$ (mg L^{-1})	DON (mg L^{-1})	pH
L. perenne (2p; LP2)	P0	0.0	0.00	3.0	17.1	0.1	4.9
L. perenne (4p; LP4)	P0	0.0	0.00	2.7	14.6	0.0	4.9
L. multiflorum (LM)	P0	0.1	0.01	1.0	3.8	0.9	5.0
F. arundinacea (FA)	P0	0.0	0.00	0.3	11.9	0.5	4.8
P. trivialis (PT)	P0	0.0	0.00	5.4	40.4	0.0	4.9
P. pratensis (PP)	P0	0.0	0.00	4.7	40.1	0.0	4.9
P. pratense (PHP)	P0	0.0	0.00	5.2	28.5	0.0	4.9
H. Lanatus (HL)	P0	0.0	0.00	0.7	10.1	0.4	5.3
L. perenne (2p; LP2)	P1	0.2	0.05	0.6	11.5	0.8	5.4
L. perenne (4p; LP4)	P1	0.2	0.06	0.3	6.7	0.9	4.9
L. multiflorum (LM)	P1	0.2	0.05	0.4	2.8	1.5	5.3
F. arundinacea (FA)	P1	0.1	0.05	0.2	9.2	0.3	5.1
P. trivialis (PT)	P1	0.1	0.04	2.2	35.8	0.0	4.9
P. pratensis (PP)	P1	0.1	0.06	3.6	38.8	0.0	4.9
P. pratense (PHP)	P1	0.1	0.04	2.4	40.3	0.0	5.0
H. Lanatus (HL)	P1	0.1	0.05	0.6	4.5	0.8	5.3

749 [a]P0 = absence of P fertilization; P1 = presence of P fertilization

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Table 4: Summary of the cumulative shoot yield, N and P uptake, and N : P ratio after eleven cuts. Per experimental treatment the mean and standard deviation are given.

ANOVA results for the two treatment factors as well as their interaction are presented per response variable.

Grass species	P treatment[a]	Yield (g pot^{-1})	N uptake (mg pot^{-1})	P uptake (mg pot^{-1})	N : P ratio (mg mg^{-1})
L. perenne (2p; LP2)	P0	46.2 ± 13.6	1938 ± 518	85 ± 26	23.9 ± 1.5
L. perenne (4p; LP4)	P0	60.1 ± 4.2	2459 ± 136	114 ± 6	22.8 ± 0.8
L. multiflorum (LM)	P0	68.6 ± 3.4	2578 ± 159	134 ± 11	20.3 ± 0.4
F. arundinacea (FA)	P0	57.6 ± 6.3	2309 ± 152	107 ± 13	23.7 ± 1.6
P. trivialis (PT)	P0	17.1 ± 3.6	807 ± 146	30 ± 5	33.4 ± 1.0
P. pratensis (PP)	P0	25.4 ± 2.5	969 ± 92	50 ± 4	22.7 ± 0.5
P. pratense (PHP)	P0	40.0 ± 10.7	1518 ± 390	71 ± 21	22.6 ± 1.0
H. lanatus (HL)	P0	72.1 ± 3.8	2680 ± 80	141 ± 9	19.8 ± 1.1
L. perenne (2p; LP2)	P1	68.1 ± 10.3	2609 ± 253	143 ± 21	18.5 ± 0.8
L. perenne (4p; LP4)	P1	76.4 ± 2.9	2909 ± 46	177 ± 8	17.8 ± 0.8
L. multiflorum (LM)	P1	79.3 ± 1.6	2777 ± 58	192 ± 8	14.5 ± 0.5
F. arundinacea (FA)	P1	71.2 ± 2.0	2635 ± 40	146 ± 6	19.8 ± 0.9
P. trivialis (PT)	P1	34.0 ± 6.6	1200 ± 360	69 ± 11	22.1 ± 0.6
P. pratensis (PP)	P1	33.4 ± 9.8	1245 ± 352	80 ± 23	16.4 ± 0.8
P. pratense (PHP)	P1	58.1 ± 11.9	2158 ± 369	128 ± 28	17.5 ± 1.1
H. lanatus (HL)	P1	85.8 ± 3.4	3106 ± 79	218 ± 12	15.1 ± 0.8
2-way ANOVA results[b]					
P treatment		***	***	***	***
Grass species		***	***	***	***
P treatment × Grass species		NS	NS	NS	***

[a]P0 = absence of P fertilization; P1 = presence of P fertilization

[b]Levels of significance for the ANOVA results: $^{*}P < 5\%$; $^{**}P < 1\%$; $^{***}P < 0.1\%$

Table 5: Root traits of roots in the topsoil and subsoil of the pots harvested after eleven cuts. Per treatment means and standard deviations are given. ANOVA results for the Two treatment factors as well as their interaction are presented per trait.

		Root mass (g)				Shoot: root ratio (g : g)		Subsoil exploration (%)[c]		Specific root length (m g^{-1})				Root length (m)				Mycorrhizal colonization (%)			
Grass species	**P treatment[a]**	**Top**		**Sub**						**top**		**sub**		**top**		**sub**		**top**		**sub**	
L. perenne (2p; LP2)	P0	4.69	± 2.28	0.41	± 0.36	9.8	± 2.4	7	± 6	423	± 26	586	± 95	2003	± 1007	226	± 201	64	± 14	48	± 27
L. perenne (4p; LP4)	P0	9.83	± 6.69	0.82	± 0.17	7.2	± 3.8	10	± 8	346	± 30	377	± 9	3273	± 1950	309	± 57	74	± 17	65	± 20
L. multiflorum (LM)	P0	11.13	± 3.90	2.21	± 0.54	5.4	± 1.6	18	± 9	333	± 71	397	± 29	3887	± 1975	866	± 151	38	± 13	66	± 1
F. arundinacea (FA)	P0	7.24	± 2.08	3.06	± 1.93	5.9	± 1.4	29	± 12	255	± 50	370	± 33	1813	± 552	1107	± 640	59	± 9	43	± 27
P. trivialis (PT)	P0	2.25	± 0.89	0.03	± 0.06	7.9	± 1.6	1	± 2	867	± 216	364	± 271	1912	± 814	22	± 39	68	± 15	21	± 18
P. pratensis (PP)	P0	2.23	± 0.37	0.07	± 0.06	11.2	± 1.1	3	± 2	759	± 65	834	± 14	1703	± 375	58	± 50	44	± 16	59	± 8
P. pratense (PHP)	P0	4.06	± 2.65	0.12	± 0.11	11.9	± 5.6	3	± 3	603	± 23	595	± 78	2409	± 1491	75	± 66	59	± 20	69	± 9
H. lanatus (HL)	P0	7.68	± 1.32	1.36	± 0.45	8.2	± 2.0	15	± 3	451	± 133	593	± 106	3421	± 1070	782	± 165	32	± 7	45	± 13
L. perenne (2p; LP2)	P1	10.39	± 6.17	0.36	± 0.22	8.2	± 5.1	3	± 1	344	± 89	459	± 98	3944	± 2974	152	± 63	33	± 14	22	± 9
L. perenne (4p; LP4)	P1	9.03	± 0.42	1.31	± 0.45	7.4	± 0.8	12	± 3	343	± 71	291	± 28	3115	± 768	388	± 166	35	± 12	41	± 18
L. multiflorum (LM)	P1	13.65	± 4.67	2.75	± 0.54	5.1	± 1.2	17	± 5	288	± 68	270	± 21	4065	± 2137	751	± 202	37	± 11	77	± 16
F. arundinacea (FA)	P1	9.61	± 0.09	2.51	± 0.65	5.9	± 0.4	21	± 4	262	± 20	311	± 63	2520	± 209	762	± 144	42	± 11	30	± 21
P. trivialis (PT)	P1	3.60	± 0.91	0.08	± 0.05	9.4	± 1.2	2	± 1	707	± 11	830	± 100	2553	± 683	69	± 40	50	± 19	28	± 13
P. pratensis (PP)	P1	1.83	± 1.00	0.08	± 0.11	20.4	± 10.6	3	± 3	746	± 124	1280	± 534	1381	± 769	146	± 219	28	± 11	12	± 3
P. pratense (PHP)	P1	5.38	± 2.70	0.15	± 0.13	12.3	± 5.5	3	± 2	614	± 179	608	± 53	3626	± 2387	94	± 81	31	± 27	34	± 9
H. lanatus (HL)	P1	6.43	± 0.92	1.78	± 0.59	10.5	± 0.8	12	± 8	449	± 39	636	± 106	2909	± 662	1174	± 589	8	± 12	11	± 4
2-way ANOVA results[b]																					
P treatment		NS		NS		NS		NS		NS		NS		NS		NS		***		***	
Grass species		***		***		***		***		***		***		NS		***		**		***	
P treatment × Grass species		NS		NS		NS		NS		NS		***		NS		NS		NS		*	

[a]P0 = absence of P fertilization; P1 = presence of P fertilization

[b]Levels of significance for the ANOVA results: $^{*}P < 5\%$; $^{**}P < 1\%$; $^{***}P < 0.1\%$

[c]Subsoil exploration was calculated as the fraction of roots that were found in the subsoil (10-40 cm)

Figure Legends:

Figure 1: Results of (a) the shoot yield (above the x axis) and the root biomass (below the x axis) per species (LP2 = diploid cultivar of *L. perenne*; LP4 = tetraploid cultivar of *L. perenne*; LM = *L. multiflorum*; FA = *F. arundinacea*; PT = *P. trivialis*; PP = *P. pratensis*; PHP = *P. pratense*; HL = *H. lanatus*) and P treatment (white bars for P0; grey bars for P1) after eleven cuts; (b) the relative shoot yield obtained per species in the absence of P fertilization, compared to the presence of P fertilization; (c), the P uptake per species and P treatment; and (d), the relative P uptake obtained per species in the absence of P fertilization, compared to P uptake in the presence of P fertilization. Within each graph, treatments with the same letter are not significantly different ($\alpha = 0.05$).

Figure 2: Trade-off relationship between root mass and specific root length in the absence (a) and presence (b) of P fertilization after eleven cuts. The symbols represent the means for the different grass species (LP2 = diploid cultivar of *L. perenne*; LP4 = tetraploid cultivar of *L. perenne*; LM = *L. multiflorum*; FA = *F. arundinacea*; PT = *P. trivialis*; PP = *P. pratensis*; PHP = *P. pratense*; HL = *H. lanatus*). The symbol size indicates the relative performance of the species in terms of shoot yield (larger symbols represent species with higher yield; see Tab. 2). The error bars represent standard errors (n = 3).

Figure 3: Results of the redundancy analysis of the pots without (a) and with (b) P fertilization after eleven cuts (n = 24 in both cases). Triangles indicate the different grass species (LP2 = diploid cultivar of *L. perenne*; LP4 = tetraploid cultivar of *L. perenne*; LM = *L. multiflorum*; FA = *F. arundinacea*; PT = *P. trivialis*; PP = *P. pratensis*; PHP = *P. pratense*; HL = *H. lanatus*) and arrows represent the different response variables for yield, P uptake (P upt), N uptake (N upt), P content (P con), N content (N con), N : P ratio (N/P), total root mass (Root M), total root length (Root L), relative subsoil exploration (Sub Exp), specific root length (SRL), shoot : root ratio (S/R), and percentage of mycorrhizal colonization (Myc Col).

Figure 4: Structural equation modeling results of plant root traits underlying the relationship with plant aboveground yield under both soil P treatments. Various root traits are used to explain the yield after eleven harvests over the various species, in the absence (a) or presence (b) of P fertilization. Black arrows represent

significant relationships (*P < 5% **P < 1% ***P < 0.1%) and gray arrows depict insignificant relationships (NS). The p-values in the bottom right indicate the likelihood that the originates from the given models (with P > 5% being significantly unlikely). In Fig. 4c the concept model defined *a priori* is given. In all figures positive and negative relationships are indicated by uninterrupted and dashed arrows respectively.

808 **Figure 1.**

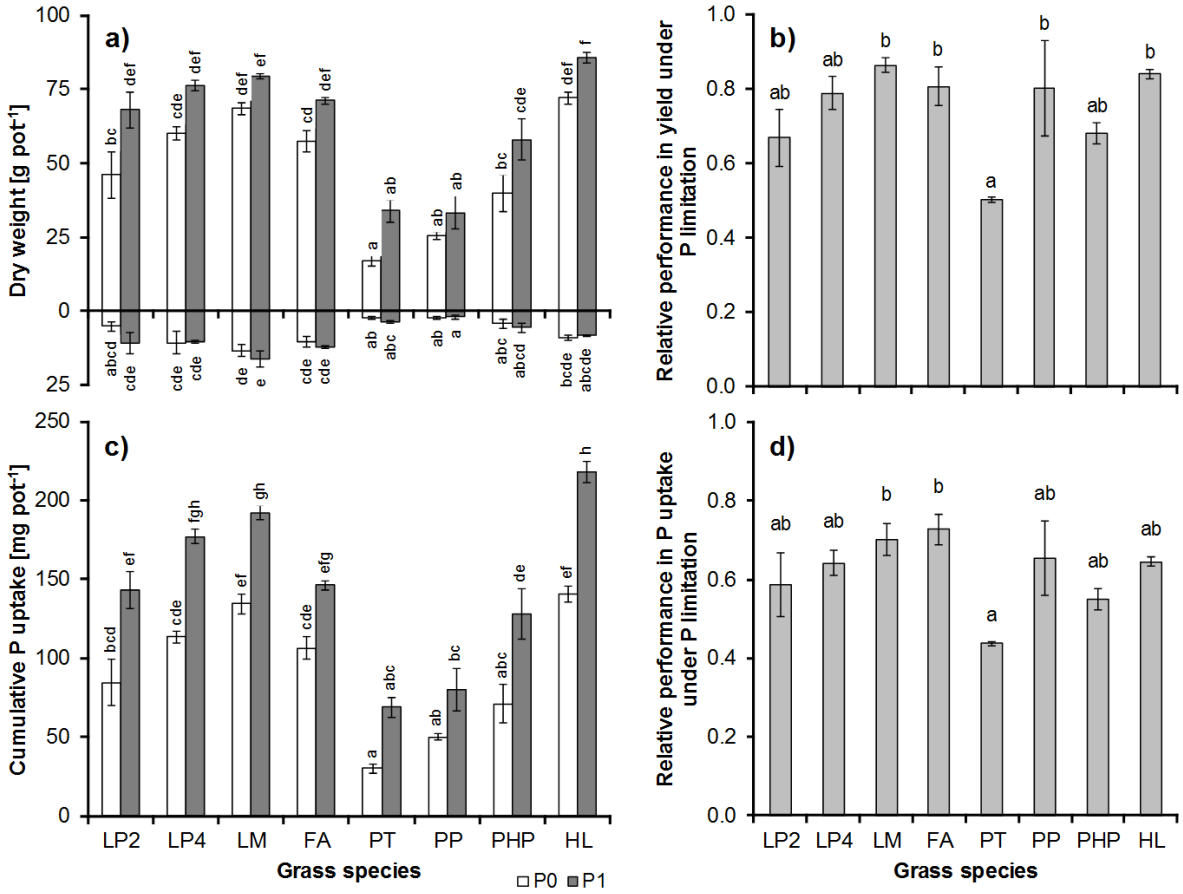

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Figure 2.

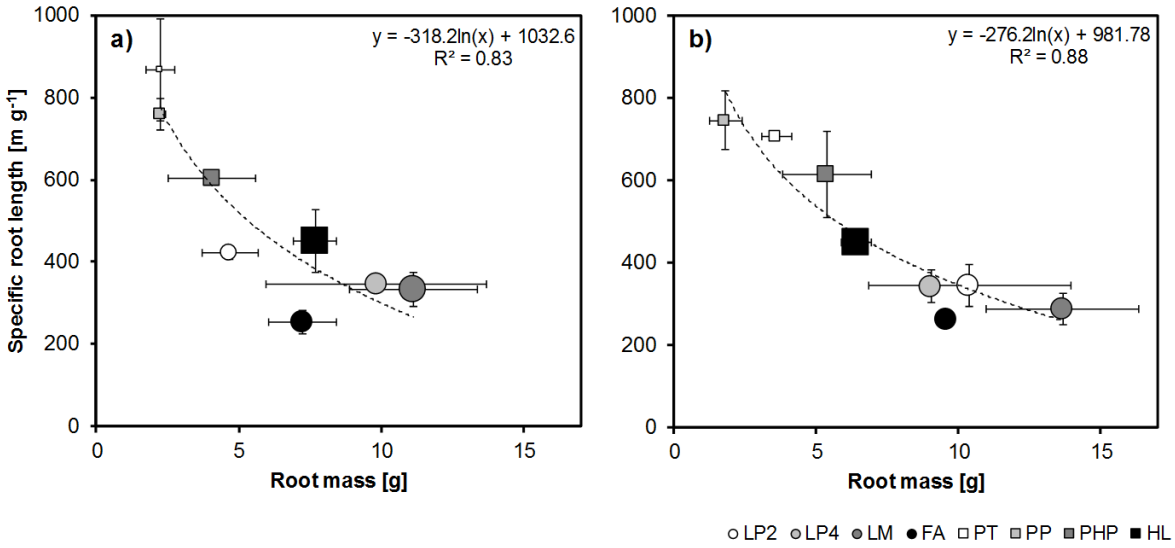

a)
y = -318.2ln(x) + 1032.6
R² = 0.83
b)
y = -276.2ln(x) + 981.78
R² = 0.88
Specific root length [m g-1]
Root mass [g]
Root mass [g]
LP2 LP4 LM FA PT PP PHP HL

Figure 3.

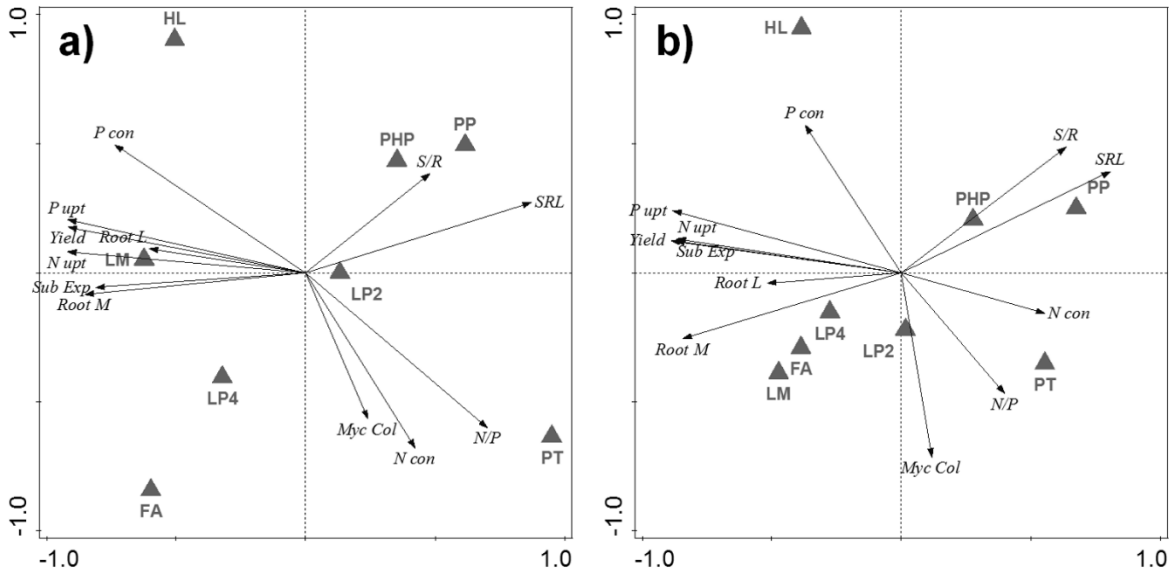
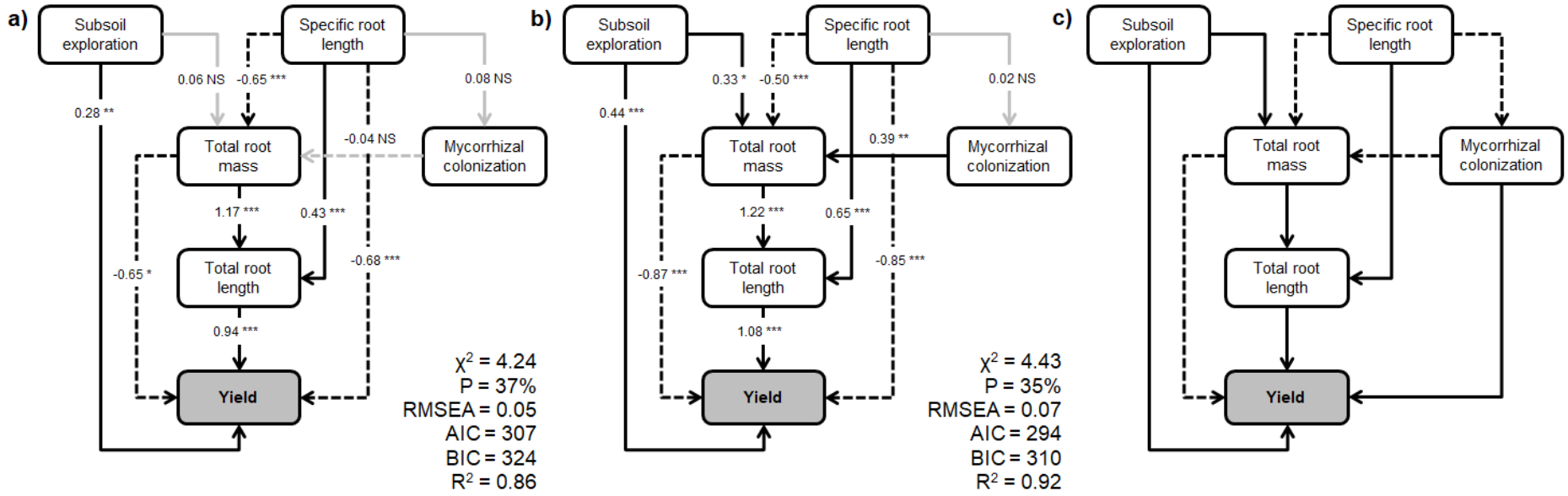

a)
HL
P con
PHP
PP
S/R
SRL
P upt
Yield
Root L
LM
N upt
Sub Exp
Root M
LP2
LP4
Myc Col
N con
N/P
PT
FA
1.0
-1.0
-1.0
1.0
b)
HL
P con
S/R
SRL
PP
PHP
P upt
N upt
Yield
Sub Exp
Root L
N con
Root M
LP4
LP2
FA
LM
PT
N/P
Myc Col
1.0
-1.0
-1.0
1.0

Figure 4.


a)
Subsoil exploration
Specific root length
Total root mass
Mycorrhizal colonization
Total root length
Yield
0.06 NS
-0.65 ***
0.08 NS
0.28 **
-0.04 NS
1.17 ***
0.43 ***
-0.65 *
-0.68 ***
0.94 ***
χ² = 4.24
P = 37%
RMSEA = 0.05
AIC = 307
BIC = 324
R² = 0.86
b)
Subsoil exploration
Specific root length
Total root mass
Mycorrhizal colonization
Total root length
Yield
0.33 *
-0.50 ***
0.02 NS
0.44 ***
0.39 **
1.22 ***
0.65 ***
-0.87 ***
-0.85 ***
1.08 ***
χ² = 4.43
P = 35%
RMSEA = 0.07
AIC = 294
BIC = 310
R² = 0.92
c)
Subsoil exploration
Specific root length
Total root mass
Mycorrhizal colonization
Total root length
Yield