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# Root growth of *Lotus corniculatus* interacts with P distribution in young sandy soil

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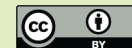
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## Abstract

Large areas of land are restored with un-weathered soil substrates following mining activities in eastern Germany and elsewhere. In the initial stages of colonization of such land by vegetation, plant roots may become key agents in generating soil formation patterns by introducing gradients in chemical and physical soil properties. On the other hand, such patterns may be influenced by root growth responses to pre-existing substrate heterogeneities. In particular, the roots of many plants were found to preferentially proliferate into nutrient-rich patches. Phosphorus (P) is of primary interest in this respect because its availability is often low in unweathered soils, limiting especially the growth of leguminous plants. However, leguminous plants occur frequently among the pioneer plant species on such soils as they only depend on atmospheric nitrogen (N) fixation as N source. In this study we investigated the relationship between root growth allocation of the legume *Lotus corniculatus* and soil P distribution on recently restored land. As test sites the experimental Chicken Creek Catchment (CCC) in eastern Germany and a nearby experimental site (ES) with the same soil substrate were used. We established two experiments with constructed heterogeneity, one in the field on the experimental site and the other in a climate chamber. In addition we conducted high-density samplings on undisturbed soil plots colonized by *L. corniculatus* on the ES and on the CCC. In the field experiment, we installed cylindrical ingrowth soil cores (4.5 × 10 cm) with and without P fertilization around single two-month-old *L. corniculatus* plants. Roots showed preferential growth into the P-fertilized ingrowth-cores. Preferential root allocation was also found in the climate chamber experiment, where single *L. corniculatus* plants were grown in containers filled with ES soil and where a lateral portion of the containers was additionally supplied with a range of different P concentrations. In the high-density samplings, we excavated soil-cubes of 10 × 10 × 10 cm size from the topsoil of 3 mini-plot areas (50 × 50 cm) each on the ES and the CCC on which *L. corniculatus* had been planted (ES) or occurred spontaneously (CCC) and for each cube separated the soil attached to the roots (root-adjacent soil) from the

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remaining soil (root-distant soil). Root length density was negatively correlated with labile P (resin-extractable P) in the root-distant soil of the CCC plots and with water-soluble P in the root-distant soil of the ES plots. The results suggest that P depletion by root uptake during plant growth soon overrode the effect of preferential root allocation in the relationship between root density and plant-available soil P heterogeneity.

## 1 Introduction

Large areas of land are denuded of the original soil cover in the course of construction or mining projects and later restored, often using un-weathered soil substrates. The formation of spatial patterns in the physical and chemical properties of the developing soil during the initial stages of colonization by vegetation is an important aspect in the restoration of such land. The development of root systems plays a particular role in these processes. Roots form pathways for water flow and solute transport and are a primary source of organic matter input into soil (Huetsch et al., 2002). Processes such as the release of organic compounds, protons and carbon dioxide, consumption of oxygen, uptake of nutrients and water can lead to steep gradients in chemical conditions and biological activities around roots, a phenomenon well known as “rhizosphere effect” (Hinsinger et al., 2005). Such gradients can have a strong influence on the patterns of mineral weathering and transformation, formation of humus, and the development of physical soil structure. Equally strong influences may also occur in the opposite direction, as pre-existing heterogeneities in soil properties can also shape the patterns of root system development. For example, many plant species are known to respond to patchiness in the spatial distribution of growth-limiting nutrients by root proliferation in patches where these nutrients are enriched (Robinson, 1994).

Limitations in the availability of soil nitrogen (N) and phosphorus (P) are a particularly frequent condition during the early phases of ecosystem development (Vitousek et al., 2010). In the absence of fertilization, mineral weathering usually is the only relevant source of P in this stage, as long as there is no major supply of P deriving from

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the decomposition of organic matter. Many pioneer plants are legumes, which do not depend on soil N, as they live in symbiosis with N-fixing rhizobia in their roots. Most of them however have high requirements for P (Sprent et al., 1988).

Phosphorus is often distributed quite heterogeneously in soil on the scale of a root system (Farley and Fitter, 1999; Gallardo and Parama, 2007; Gross et al., 1995; Jackson and Caldwell, 1993). Laboratory and greenhouse experiments with constructed heterogeneities and/or split root systems have shown that localized P supply can induce preferential root proliferation in many plant species (Kume et al., 2006; Ma and Rengel, 2008; Ma et al., 2007; Robinson, 1994; Weligama et al., 2007). Some authors also studied preferential root growth in response to localized P fertilization in the field (Eissenstat and Caldwell, 1988; Buman et al., 1994; Caldwell et al., 1996). In studies with artificially created heterogeneity the contrast in P concentrations between fertilized and non-fertilized soil patches was usually high. Little is known about the extent and relevance of preferential root growth in response to P patchiness under normal field conditions. Mou et al. (1995) analyzed three-dimensional root distributions in monocultural Sweetgum Sprout and Loblolly pine plantations in relation to available soil P, K and N concentrations and found that the fine root densities of both tree species increased with P and K but not N concentrations in the topsoil.

In this study we had the opportunity to investigate the root allocation strategy of the legume *Lotus corniculatus* in the man-made 6-ha Chicken Creek Catchment (CCC), which was established in 2005 in a Lusatian post-mining landscape in Eastern Germany to study initial ecosystem development on freshly deposited non-weathered substrate on a catchment scale (Gerwin et al., 2009). *Lotus corniculatus* L. (bird's foot trefoil) is a perennial herbaceous early-succession plant pioneering the colonization of post-mining landscapes in Lusatia.

On the catchment we sampled roots and soil at high-density on 3 mini-plot areas where *L. corniculatus* occurred spontaneously. Because disturbances in general and erosion risks in particular had to be kept at a minimum on the CCC, an experimental site (ES) with similar soil properties was established in vicinity of the CCC, where soil and

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vegetation could also be experimentally manipulated. On this site we carried out the same mini-plot high-density sampling as on the CCC, but after growing *L. corniculatus* in monoculture. In addition, we performed a factorial plot experiment on this site and a climate chamber experiment with constructed heterogeneities to test the response of *L. corniculatus* to P-enriched soil also under more controlled conditions.

We expected that soil patches with elevated concentrations in P would induce preferential root allocation and that we would therefore find a positive correlation between root length density and soil P in the high-density samplings.

## 2 Materials and methods

### 2.1 Site description

The Chicken Creek Catchment (CCC) was constructed on a refilled open cast lignite mine about 30 km south of the city of Cottbus in the State of Brandenburg, Germany. After construction was finished in September 2005, the site was left to re-vegetate spontaneously. A detailed description of the establishment and initial development of the catchment was given by Gerwin et al. (2009). In order to enable also manipulative and invasive field experiments with soil and plants under comparable conditions, the before-mentioned “Experimental Site” (ES) was established in 2009 in the vicinity of the CCC using substrate of the same origin.

The substrate deposited on the CCC and the ES as soil parent material was quarternary calcareous sand from Saale-time Pleistocene deposits of the Lusatian ridge (in German: Lausitzer Höhenrücken). The soil parameters of the substrate on the ES and the CCC are illustrated in Table 1. Soil parameters for the CCC derive from a soil sampling campaign conducted in 2005 (Gerwin et al., 2009) and are averaged values of sampling points proximate to the investigated plots, while soil parameters for the ES represent values taken from soil sampled at the investigated plots.

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The climate is temperate and slightly continental with high summer temperatures and pronounced drought periods during the growing season. The long-term average precipitation was given as 595 mm per year, and the mean air temperature as 9.3 °C (Gerwin et al., 2009). The main difference between the two sites was that the CCC was built as a large lysimeter with an impermeable clay liner at 2–3 m depth in order to collect all water at the catchment outflow, while there was free drainage from the ES soil. Consequently, a water table developed in the subsurface of the CCC in contrast to the ES, and as the hydraulic conductivity of the deposited substrate was lower than predicted, the water table rose to higher levels than planned, and at times some water even influenced the lower parts of the root zone.

## 2.2 Climate chamber experiment

The climate chamber experiment was performed at ETH Zürich. Single *L. corniculatus* plantlets were grown in Al-containers of 27 × 27 × 1.2 cm internal volume (height × width × depth) filled with soil from the experimental site. We established 6 homogeneous soil treatments adding 5.7, 17, 34, 52, 85 or 102 mg P per pot (4, 12, 24, 37, 60 72 mg P kg<sup>-1</sup> soil) and 8 heterogeneous soil treatments. In the latter we added 5.7, 11.3, 17, 34, 51, 68, 85 or 102 mg P pot<sup>-1</sup>, but only to a lateral third of the soil in each container (12, 24, 36, 72, 109, 145, 182, 218 mg P kg<sup>-1</sup> soil in the P-enriched soil area). Additionally, we established a control treatment with no P addition. All treatments were replicated three times, except for the highest heterogeneous treatment, which was replicated only twice. Mono-calcium-phosphate (Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>·H<sub>2</sub>O) was used as fertilizer.

To fill the soil into the containers, we laid them down on one side and removed the upward looking lateral wall of the other side. Then the soil, which had been thoroughly mixed with respective amounts of fertilizer before, was filled in three vertical bands of equal width (9 × 27 cm) into the containers. In the heterogeneous treatments, we always filled the P fertilized soil into the third on the right-hand side looking into the opened container. After filling we closed the lateral wall and put the container into the upright

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position again. Care was taken to avoid pressing of the soil and to achieve a dry soil bulk density of approximately  $1.6 \text{ g cm}^{-3}$  in all containers.

We planted a single pre-germinated seedling in the middle of each container, so that the distances to the left and the right compartment were the same. Plants were grown for 60 days in a climate chamber with a humidity of 60 %, a 16 : 8 h day : night cycle and a respective 21/16 °C temperature cycle. During the day the photon flux was  $250 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . We watered the container on a weight basis to 50 % water holding capacity (approx. 100 hPa water suction).

At harvest, we cut the shoots close to the soil surface and dried them to constant weight at 60 °C. The roots were sampled separately from each third of the containers. After thoroughly washing the soil from the roots, they were placed into a water bath and scanned with an Epson scanner (perfection V700, 400 dpi resolution). The scans were then analyzed for root length by means of WinRHIZO (Regent Instruments, Inc. Quebec Canada, version 2009a).

### 2.3 Ingrowth core experiment

We used the ingrowth core method for the factorial plot experiment with constructed P heterogeneities on the ES. Single *L. corniculatus* plantlets were grown on 18 plots of  $50 \times 50 \text{ cm}$  size, on which fertilized (as described below) and non-fertilized soil cores were installed in a regular grid at distances of 10, 22 and 33 cm from the plant stem in the center of each plot. Additionally, we established plots with homogeneous P fertilization (applying the same P rate as to the soil of the fertilized cores in the other plots) of the entire topsoil and non-fertilized control plots in order to assess the potential P responsiveness of *L. corniculatus* on the experimental site.

To prepare the plots for planting, we excavated and bulked the entire topsoil (0–10 cm) of all plots, homogenized it thoroughly and divided it into two fractions. One fraction was mixed with  $27 \text{ mg P kg}^{-1}$  soil, while the other fraction remained unfertilized. At first, the ingrowth cores were established using steal cylinders of 10 cm height and 4.5 cm diameter placed in upright position on a  $20 \times 20 \text{ cm}$  square grid. As the

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center of the plot was aligned with the center of the central square, his scheme resulted in 4, 8 and 4 ingrowth cores at 10, 22 and 33 cm distance from the center of the plot, respectively. Alternatively, half of the cylinders were filled with fertilized and unfertilized soil. After re-filling the space around the cylinders with unfertilized soil, the cylinders were carefully removed. Similarly, just without previous ingrowth core installation, homogenized soil with or without fertilization was filled back into the plots of the respective homogeneous treatments. Each treatment was replicated six times.

Two month before the experiment started, we sowed *L. corniculatus* seeds on the ES to establish a pool of candidate plantlets. From this pool we selected plantlets of similar size and habitus and transplanted them on 15 April 2009 to the experimental plots All plots were weeded once weekly. On 1 October 2009, we harvested the shoots and sampled all ingrowth cores. After transfer to the laboratory, the roots were processed and analyzed in the same way as in the climate chamber experiment.

## 2.4 High density sampling on the Chicken Creek Catchment and the Experimental Site

After manual removal of existing plants, three otherwise undisturbed 50 × 50 cm mini-plots were seeded with *L. corniculatus* in fall 2008 at low, medium and high density, as specified in Table 2. Keeping the plots clean from other plants also was the only manipulation of the plots during the growth of the *L. corniculatus* seedling. In spring 2009 the plants were harvested and the soil collected in 10 × 10 × 10 cm cubes. The same type of sampling was performed on three mini-plots of the same size in May 2010 on the CCC, with the difference that in contrast to the ES plots *L. corniculatus* was present on these plots spontaneously. While plots were selected which were predominantly but sparsely populated with *L. corniculatus*, it was unavoidable that also other plants – exclusively grass species – were present as well.

The soil cubes were collected by means of metal boxes, which were driven side by side into the soil (25 cubes per plot). The samples (containing soil and roots) were transferred into plastic bags and immediately transported in thermo boxes into the

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laboratory, where they were stored in a refrigerator at 4 °C, until they were further processed and analyzed within the following 1–4 weeks. Roots with adherent field-moist soil were separated from the remaining soil, in the following referred to as *root-distant soil*, by means of a 4 mm sieve. Grass roots in the CCC samples were easily distinguished and separated from *L. corniculatus* roots. Grass roots and the soil attached to these roots was excluded from soil or root analysis. The soil adhering to the roots, in the following referred to as *root-adjacent soil*, was left to air-dry for 5 min and then gently removed using a brush. *Root-adjacent* and *root-distant soil* samples were stored separately in small parchment paper bags for subsequent chemical analyses. After thorough washing, the roots the roots were analyzed in the same way as in the experiments described before.

Water soluble P and calcium (Ca) concentrations and the pH of root-distant and root adjacent soil samples were analyzed in 1 : 2.5 soil-to-solution extracts, using bi-distilled water for extraction (Meiwes et al., 1984). After adding the water, the slurries were shaken for 1 h and then left to settle for 16 h at room temperature, centrifuged for 5 min at 3000 rpm and filtered (512 ½ folding filter, Whatman; Dassel, Germany) following the method of Schlichting et al. (1995). The filtrates were analyzed for Ca and P by means of inductive coupled plasma spectrometry (iCAP 6000 series, Thermo scientific, Germany). The CCC samples were also analyzed for anion- and cation-resin extractable P using the method of Saggar et al. (1990). Phosphorus concentrations in solution were determined photometrically (Van Veldhoven and Mannaerts, 1987). In the following we refer to the resin-extractable P as labile P.

### 2.5 Statistical analysis and calculations

We used normal quantile-quantile plots to check for deviations from normal distribution of random effects and residual errors. The labile P, water-soluble P and root length data from the CCC samples were log-transformed to achieve normality. In all other cases no transformation was necessary.

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In the climate chamber experiment, we calculated root allocation as the difference of root length in the right third of the container (fertilized in the heterogeneous treatments) and the left third (unfertilized in the heterogeneous treatments). We used the protected Fisher LSD test for multiple comparisons. If the lower boundary of the 95 % confidence interval was greater than zero, root allocation was considered preferential.

The datasets of the high-density samplings were analyzed separately for the two sites. We standardized root length and soil parameters by plots to achieve mean values of 0 and variances of 1 for all parameters on each plot. Then we pooled the standardized data of the three plots of each site and calculated Pearson correlation coefficients of the soil parameters, distance from the stem and root length. Distance from the stem of a sampled cube was calculated as the distance from the center of the cube containing the nearest plant and the center of the cube in question. Rhizosphere effects for labile P, water-soluble P, Ca and pH were determined as the difference between concentrations of the root-adjacent and root-distant soil in a cube sample.

### 3 Results

#### 3.1 Climate chamber experiment

The growth habitus of the experimental plants showed considerable variation in the climate chamber experiment, indicating substantial genotypic variability among the seeds. As a result, neither heterogeneous nor homogeneous P fertilization showed a significant influence on shoot dry-weight production (ANOVA,  $p < 0.05$ ). At low P supply shoot biomass tended to increase with increasing level of fertilization (Fig. 1a).

In the heterogeneous treatments, root length was always significantly higher in the P-fertilized part of the containers than in the unfertilized part (Fig. 1b). In the homogeneous treatments, root length, as to be expected, did not significantly differ between the two sides of the containers. Despite the large variability in plant growth, the experiment thus revealed a clear preferential root growth response to increased P concentration.

## 3.2 Ingrowth core experiment

As we selected the plants according to their size, growth habitus and leaf shape for the ingrowth core field experiment, it can be assumed that they were genetically much more homogeneous than in the climate chamber experiment. Fresh-weight production of the shoot biomass was 2.5 times higher in the homogeneous P fertilization treatment than in the ingrowth core and control treatments (Fig. 2a).

Root length was larger in the P fertilized ingrowth cores than in the unfertilized cores (2-way ANOVA,  $p < 0.05$ ) and decreased with increasing distance from the stems of the plants (2-way ANOVA,  $p < 0.05$ ). As Fig. 2b shows, the effect of P on root length production was strongest close to the plant stems and decreased with distance. The gradient of decrease appeared to be larger for the P fertilized cores than the control cores, but the interaction between P fertilization and distance from the stem was not significant.

## 3.3 High density samplings

Root length density did not significantly change with distance from the stems on the CCC plots (Table 4), while it decreased with increasing distance from the stems on the ES plots (Table 3, Fig. 3). Root length density also decreased with increasing water-soluble P on the ES plots, while no correlation between these two variables was found on the CCC plots. A similar negative relationship as between water-soluble P and root length density on the ES was found between labile P and root length density on the CCC (Fig. 4). In contrast to the finding that the relationships of root length density with stem distance and water-soluble P were both negative, water-soluble P was not affected by stem distance on the ES plots. But distance from the stem had a positive effect on labile P on the CCC, while it showed no influence on water-soluble P. Soil Ca concentration increased with root length density on the ES plots and decreased with distance from the stem. Soil pH and Ca were negatively correlated on both sites. Furthermore, pH increased also with distance on the CCC, but not on the ES.

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Water-soluble Ca was in average 28 mg kg<sup>-1</sup> higher in root-adjacent soil than in root-distant soil on the ES (Fig. 5), while the pH of root adjacent soil was in average 0.4 units lower than the pH of root-distant soil. A similar but weaker rhizosphere effect on water-soluble Ca as in the ES soil was also found in the Chicken Creek samples, whereas no consistent effect on pH was detected. While we found no significant rhizosphere effect on water-soluble P in ES soil, it tended to be higher in root-adjacent than in root-distant soil for all three plots. In contrast to this trend, water-soluble P concentrations tended to be slightly lower in root-adjacent than in root-distant Chicken Creek soil. On the other hand, labile P was higher in root-adjacent than in root-distant soil of the Chicken Creek plots, similarly to the rhizosphere effect on water-soluble P of the ES soil.

#### 4 Discussion

The results of the fertilization experiment on the ES area clearly show that low soil P was limiting the growth of *L. corniculatus* in the unfertilized soil and that *L. corniculatus* responds with root proliferation into P-enriched soil under these conditions. The climate chamber experiment, where all other heterogeneities had been evened out by soil homogenization, confirmed that preferential allocation of root growth is indeed a response of *L. corniculatus* that can be induced by heterogeneous P. distribution. The ability to respond to locally increased P availability with enhanced root proliferation has been shown also for many other plant species (Ma and Rengel, 2008; Ma et al., 2007; Kume et al., 2006; Robinson, 1994; Weligama et al., 2007) in climate chamber experiments, but seldom in the field (Eissenstat and Caldwell, 1988; Buman et al., 1994).

The negative correlations of root length density with labile P and water-soluble P in the root-distant soil on the high-density sampling plots of the two field sites is in direct contrast to the results of the experiments with constructed heterogeneity. They suggest that plant-available soil P was quite rapidly depleted by root uptake and that this depletion had a stronger influence than preferential root proliferation into P-rich soil on the relationship between root length density and soil P at the time of sampling.

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Furthermore, it indicates that the influence of the roots extended into zones around the roots beyond our operationally defined root-adjacent soil. The negative correlation between root length and labile and water-soluble P is also in contrast to the results of Mou et al. (1995), who found a positive correlation between root length growth and soil P in Loblolly Pine and Sweetgum monocultures. However, in contrast to our sites, these stands were already in a stage of ecosystem development in which P recycling with root and shoot litter decomposition probably is a major process determining P distribution in soil. Recycling of P by litter decomposition could result in high contrasts between P-rich and P-poor patches, as P is extracted from the entire volume of soil colonized by roots, but the relation with necromass decomposition would be concentrated according to the mass of the decaying roots.

Comparing the results of the high-density samplings with those of the experiments with constructed heterogeneities, it must be considered that the variation in plant-available soil P was in average much smaller in the undisturbed field soil than the contrasts between fertilized and non-fertilized soil in the latter experiments. Furthermore, the plants sampled on the CCC had much more time to develop their root systems and extract soil P than in the ingrowth core and the climate chamber experiment. Thus, it is quite plausible that preferential root allocation into initially P-rich occurred, but was subsequently masked by the opposite effect of P depletion. It is also conceivable that P heterogeneity in the undisturbed field soils was too small to trigger preferential root growth allocation in P-enriched soil zones. Several authors investigating root distributions in relation to soil nutrient distributions suggested that P heterogeneity in their study soils was too low to become relevant for root allocation in herbaceous plants, but not for trees (Farley and Fitter, 1999; Gallardo and Parama, 2007; Gross et al., 1995).

In apparent contrast to the notion that P becomes depleted with time in the rhizosphere (Hendriks et al., 1981; Hinsinger et al., 2011b; Wang et al., 2005), we observed elevated concentrations of labile P fractions in soil adjacent to roots as compared to soil farther away from the roots in the high density samplings. Likely reasons for this effect are P solubilization by root exudation and rhizosphere acidification (Hinsinger et

al., 2011b). Given that P is a rather immobile nutrient element in soil, the direct influence of roots on P concentrations only extends a few mm at most into the adjacent rhizosphere soil (Hinsinger et al., 2011b). By exudation of organic acids such as citric acid, which diffuse into the surrounding rhizosphere soil and mobilize phosphate from solid phases, plants can substantially increase the flux of soil P to their roots. Such solubilization can result in higher average concentrations of labile or water-soluble P in the rhizosphere than in the bulk soil, even when the total P concentration is reduced and despite a concentration gradient in dissolved P towards the root surface. Support for this interpretation comes from findings of P depletion in the rhizosphere immediately adjacent to the root surface and P enrichment above bulk soil level in the outer zone of the rhizosphere just a few mm farther (Hinsinger and Gilkes, 1996; Hinsinger et al., 2011b; Hubel and Beck, 1993).

Whether accumulation or depletion of P is found in the rhizosphere, thus, may also depend on the extent to which soil adjacent to the root surface is included in “rhizosphere” soil samples and explain why some authors found depletion of P and others accumulation of P in the rhizosphere. (Hinsinger et al., 2011a) suggest that the interaction of P uptake rate and P solubilization through exudates are responsible for P concentration pattern.

While lower water-soluble or labile P concentrations in root-distant than in the root-adjacent soil can be explained by P solubilization through root exudates, the rhizosphere effect does not explain the negative correlation observed between root density and labile or water-soluble P in the root-distant soil. A likely candidate would be soil P extraction via arbuscular mycorrhizal fungi (AMF). Extraradical mycorrhizal hyphae can grow far beyond the zone directly influenced by the roots and extract P from soil up to 10 cm away from the root surface (Jansa et al., 2005). Mycorrhizal fungi can contribute much more than direct root uptake to the P nutrition of plants. Smith et al. (2004) for example showed that 50 to 100 % of the P accumulated in the shoots of three plant species was taken up via mycorrhizal fungi. If the density of extraradical mycorrhizal hyphae was positively correlated with root length density and root age, then this could

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explain that P depletion increased with root length density in the root-distant soil. Indeed we found that the roots of three randomly selected *L. corniculatus* plantlets were colonized with AMF in the climate chamber experiment.

While the positive rhizosphere effect on labile P in the CCC plots was in line with the corresponding effect on water-soluble P in the ES plots, it was surprising that the rhizosphere at the same time had the opposite effect on water-soluble P, a fraction closely related to labile P, in the CCC soil. This puzzling result may be explained by the different water regimes of the two sites and their effect on soil carbonate dynamics. The CCC was under the influence of a fluctuating groundwater table in the subsoil, in contrast to the ES. At times, the water table was high enough that through the capillary fringe above the water table even topsoil roots could probably tap into this source during some periods. Thus, the vegetation on the CCC plots could consume much more water than on the ES, and this transpirational water stream could result in a substantial upward flow of calcium carbonate saturated solution from the groundwater table to the roots at certain times. Calcium supplied in excess of plant uptake (Hinsinger et al., 2005). would have accumulated in the rhizosphere and eventually precipitated as  $\text{CaCO}_3$ , in particular when the partial pressure of  $\text{CO}_2$  decreased during drying phases. Thus, the pH buffer capacity provided by  $\text{CaCO}_3$  was periodically replenished in the rhizosphere of the CCC plots, maintaining pH at similar or even higher levels as in the bulk soil and keeping water-soluble P at correspondingly low levels. In contrast, as the buffer was gradually depleted, pH values decreased and water-soluble P concentrations increased in the rhizosphere of the ES plots (Fig. 4). The fact that, unlike the concentration of water-soluble P, the concentration of resin-extractable P was higher in the rhizosphere than in the bulk soil of the CCC plots suggests that a comparatively large fraction of this P had been mobilized from less available P pools by root exudates and bound in labile, but not water-soluble form, e.g. on ion-exchanging sites.

The effect of stem distance on root length density differed between the one-year-old plants on the ES and plants on the CCC plots, which were in average older than one year, indicating that new root growth was preferentially allocated at greater distances

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from the stems with plant age. This could be a response to increasing nutrient depletion in soil around older parts of a root system, as long as zones farther away are still more abundant in nutrients and water. Indeed, we found a decrease of labile P in the root-distant soil with distance from stems on the CCC. Most authors investigating herbaceous plants or grasses found that root length density decreased with distance from stem after one growing season (Buman et al., 1994; Majdi et al., 1992; Milchunas et al., 1992). But they did not study perennial growth. For trees, some authors found that within the sampled range the distance from the stem had no influence on root length density (Millikin and Bledsoe, 1999). In line with our observations, these findings suggest that an initial dependence of root density on stem distance disappears with plant age.

## 5 Conclusions

The experiments with constructed heterogeneity clearly showed that *L. corniculatus* preferentially allocated roots into P-enriched soil zones in the low P soils of this study. The results of the high-density samplings on the other hand indicate that P depletion by roots and probably also mycorrhizal fungi had a more dominating influence on the spatial relationship between root length density and soil P concentrations in the field soil without constructed P heterogeneity. Assuming that also *L. corniculatus* plants grown on CCC and unfertilized ES soil preferentially allocated roots into P-enriched soil zones, this means that depletion of these regions by root P uptake subsequently turned these soil areas into patches with decreased P availability compared to the surrounding. While the combined effect of preferential root growth and soil P depletion by roots is expected to reduce contrasts between soil patches of higher and lower P-availability during the initial stages of soil development, other processes may oppose this trend by the generation of new and even stronger heterogeneities, in particular locally concentrated P inputs with leaf and root litter.

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**Table 1.** Soil parameters of the Experimental Site (ES) and the Chicken Creek Catchment (CCC).

	Sand	Silt	Clay	Organic carbon (%)	Calcium carbonate (%)	pH (H <sub>2</sub> O)
ES	96.3	1.6	2	<0.01	0.82	8.50
CCC	86.0	8.0	6	0.14	0.65	8.07

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**Table 2.** Number of *L. corniculatus* plants and coverage (%) per plot (50 × 50 cm) at the experimental site (ES) and Chicken Creek Catchment (CCC) for low (plot 1), intermediate (plot 2) and high (plot 3) vegetation density. For the coverage of plot 2 and plot 3 on the CCC numbers in brackets refer to the coverage of *L. corniculatus* plus the co-occurring grass species.

	Plot 1 ES	Plot 2 ES	Plot 3 ES	Plot 1 CCC	Plot 2 CCC	Plot 3 CCC
Number of plants per plot	6	7	9	1	3	6
Coverage (%)	16	36	48	16	44 (90)	48 (100)



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**Table 3.** Pearson correlation coefficients (lower part of the table) and p-values (upper part of the table) for root length, distance from the stem, water-soluble Ca, pH and water-soluble P on the Experimental Site (ES). The numbers in italic indicate significant correlation between two variables ( $p < 0.05$ ).

	Root length	Distance	Water-sol. Ca	pH	Water-sol. P
Root length		<i>0.0034</i>	<i>0.0000</i>	0.2437	<i>0.0207</i>
Distance	<i>-0.35</i>		<i>0.0009</i>	0.2607	0.6949
Water-sol. Ca	<i>0.6</i>	<i>-0.44</i>		<i>0.0206</i>	0.6859
pH	-0.14	0.14	<i>-0.32</i>		<i>0.0283</i>
Water-sol. P	<i>-0.28</i>	-0.05	-0.06	<i>0.27</i>	

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**Table 4.** Pearson correlation coefficients (lower part of the table) and p-values (upper part of the table) for root length, distance from the stem, water-soluble Ca, pH and water-soluble P on the Chicken Creek Catchment (CCC). The numbers in italic indicate significant correlations between two variables ( $p < 0.05$ ).

	Root length	Distance	Water-sol. Ca	pH	Water-sol. P	Labile P
Root length		0.1512	0.7251	0.4737	0.3016	0.0038
Distance	-0.17		0.0372	0.0256	0.1762	0.0066
Water-sol. Ca	-0.04	-0.24		0.0004	0.0000	0.0584
pH	0.08	0.26	-0.40		0.0783	0.7651
Water-sol. P	-0.12	0.16	-0.46	0.21		0.0004
Labile P	-0.33	0.31	-0.22	0.03	0.41	

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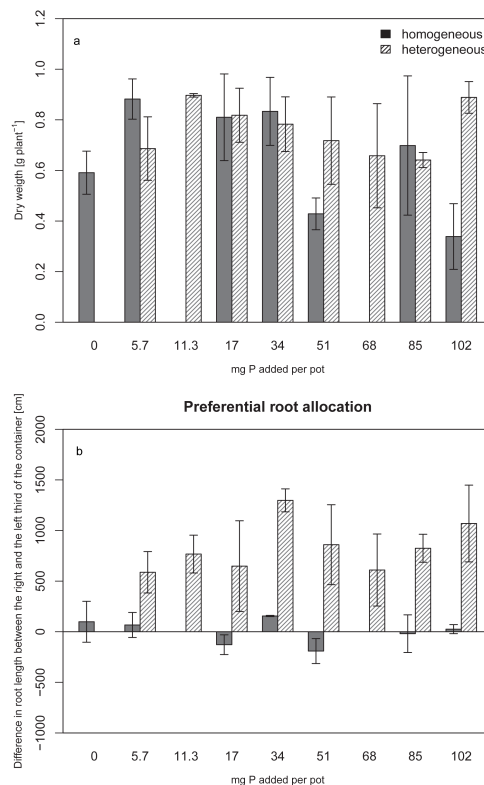
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**Fig. 1. (a)** Shoot dryweight production of *L. corniculatus* grown in containers filled heterogeneously (stippled bars) or homogeneously (grey bars) with soil. **(b)** Preferential root allocation was calculated as the difference of root length in the right third of the container (P fertilized in the heterogeneous treatments) and the left third of the container (unfertilized). Error bars refer to the standard error of the mean. Preferential root allocation was significant in all heterogeneous treatments.

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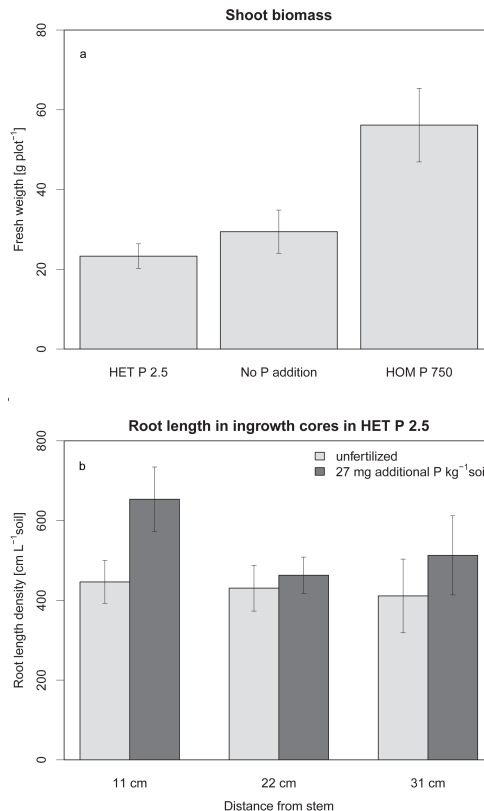
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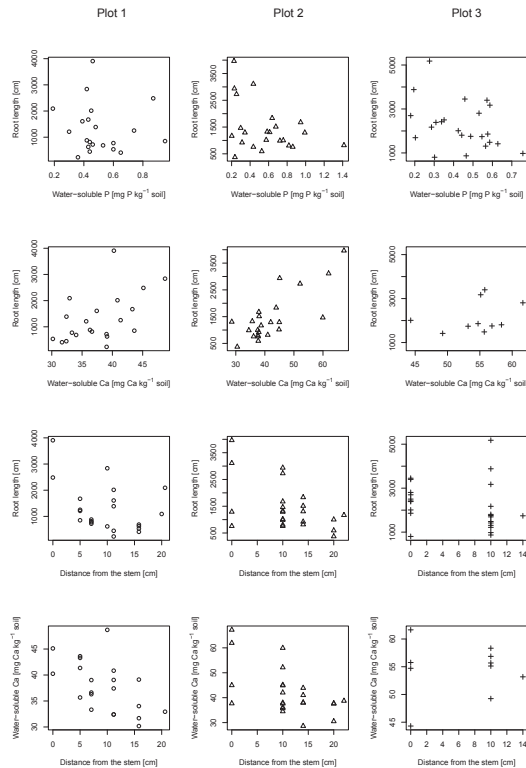




**Fig. 2. (a)** Shoot fresh weight production of single field-grown *L. corniculatus* plants grown on plots with heterogeneous P fertilization (HET P 2.5), no P addition (No P addition) or homogeneous P supply (HOM P 750). **(b)** Root length density in fertilized and unfertilized ingrowth cores of HET P 2.5 at 11, 22 and 31 cm from the stem of the plants. Error bars refer to the standard error of the mean. P fertilization and distance had a significant effect on root length density in the ingrowth cores (2-way-ANOVA,  $p < 0.05$ ).

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**Fig. 3.** The relationship between root length and water-soluble P (first row) or water-soluble Ca (second row) as well as the relationship between the distance from the stem and root length (third row) or water-soluble Ca (fourth row) investigated in the high density sampling on the Experimental Site (ES) in the top-soil (0–10 cm) of the plots with low (plot 1), intermediate (plot 2) and high vegetation density (plot 3).

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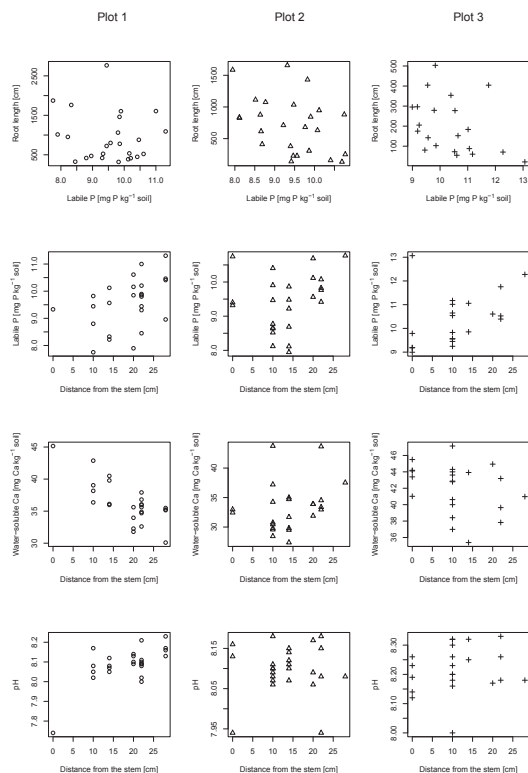
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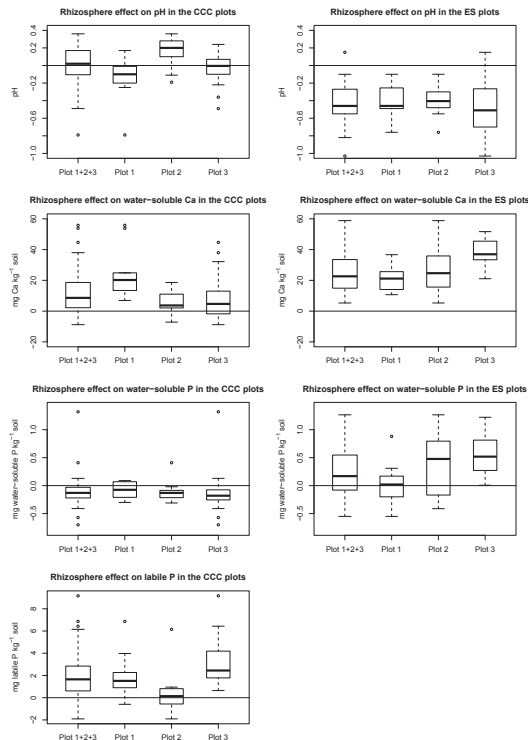
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**Fig. 4.** The relationship between root length and labile P (first row) as well as the the relationship between the distance from the stem and labile P (second row), water-soluble Ca (third row) or pH (fourth row) investigated in the high density sampling on the Chicken Creek Catchment (CCC) in the top-soil (0–10 cm) of the plots with low (plot 1), intermediate (plot 2) and high vegetation density (plot 3).

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**Fig. 5.** The rhizosphere effect for pH, water-soluble Ca, water-soluble P and labile P on the plots with low (plot 1), intermediate (plot 2), high (plot 3) vegetation density and the pooled data for the three plots (plot 1 + 2 + 3) on the Chicken Creek Catchment (CCC) and the Experimental Site (ES) is calculated as the difference between the value for the respective parameter in the soil attached to the root and the value in the remaining soil of cubic samples taken from the top 10 cm of the soil profile. Boxplots illustrate the median (horizontal line), the interquartile range (box), 1.5 times the interquartile range (whiskers) and outliers.