



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

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**Abstract.** Large areas of land are restored with unweathered 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 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, and uptake of nutrients and water can lead to steep gradients in chemical conditions and biological activities around roots, a phenomenon well known as the “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 is usually the only relevant source of P in this stage, as long as there is no major supply of P deriving from 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; Denton et al., 2006). 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 *Liquidambar styraciflua* and *Pinus taeda* 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 with N concentrations in the topsoil. These stands were already in a later stage of ecosystem development, at which root, shoot and leaf litter decomposition already may have played a major role for the spatial distribution of soil nutrients. We are not aware of studies that investigated the effect of heterogeneous soil nutrient distribution on root allocation patterns in soils in the initial stage of ecosystem development and which compared the response of roots to nutrient-enriched soil patches under experimental conditions with the

relationship between root allocation and soil nutrient distribution under undisturbed conditions in the field.

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 (in German: Lausitz) 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 the vicinity of the CCC, where soil and 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, opencast 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 aforementioned “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 quaternary 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

**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

soil parameters for the ES represent values taken from soil sampled at the investigated plots.

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 Aluminium 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 position again. Care was taken to avoid pressing of the soil, and to achieve a

dry soil bulk density of approximately 1.6 g cm<sup>-3</sup> in all containers.

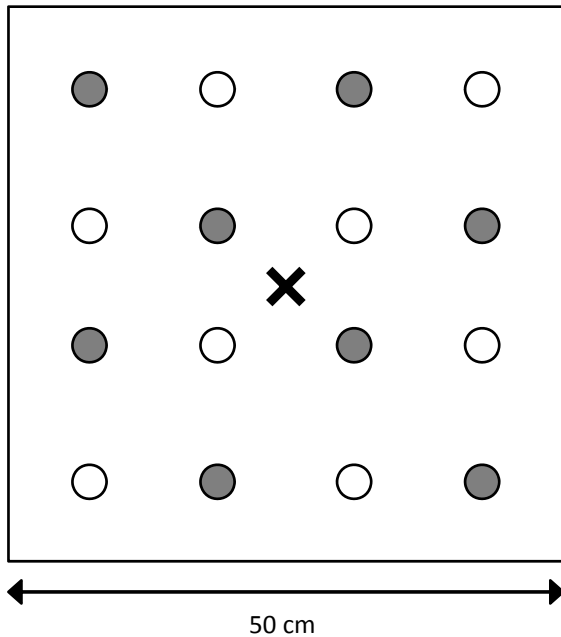
We planted a single pre-germinated seedling in the middle of each container, so that the distances to the left and the right compartment were the same. Plants were grown for 60 days in a climate chamber with a humidity of 60 %, a 16 : 8 h day : night cycle and a respective 21/16 °C temperature cycle. During the day the photon flux was 250 μmol m<sup>-2</sup> s<sup>-1</sup>. 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

For the factorial plot experiment on the ES we employed the ingrowth core method. Single *L. corniculatus* plantlets were grown on 18 plots of 50 × 50 cm size, on which fertilized (as described below) and non-fertilized cylindrical soil cores were installed vertically on a regular grid at distances of 10, 22 and 30 cm from the plant stem in the center of each plot (Fig. 1). The total rate P applied was 55 mg per plot. Thus, this treatment is denoted here as HET P 55. Additionally, we established plots with homogeneous P fertilization of the entire topsoil (0–10 cm depth) and non-fertilized control plots in order to assess the potential P responsiveness of *L. corniculatus* on the experimental site. The rate of P application in the homogeneous fertilization treatment was the same as for the fertilized cores in the HET P 55 treatment, resulting in a total rate of 1080 mg P applied per plot. This treatment thus is denoted here as HOM P 1080.

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 mg P kg<sup>-1</sup> soil (as monocalcium phosphate), while the other fraction remained unfertilized. At first, the ingrowth cores were established using steel cylinders of 10 cm height and 4.5 cm diameter placed in upright position on a 14 × 14 cm square grid. As the center of the plot was aligned with the center of the central square, its scheme resulted in 4, 8 and 4 ingrowth cores at 10, 22 and 30 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



**Fig. 1.** Positioning of P-fertilized (grey circles) and unfertilized soil cores (unfilled circles) around single *L. corniculatus* plants (cross) on plots (50 × 50 cm) with heterogeneous P supply of the ingrowth core experiment. Soil cores were arranged on a 14 × 14 cm grid resulting in 4, 8 and 4 soil cores at 10, 22 and 30 cm distance from the plant, respectively.

plots of the respective homogeneous treatments. Each treatment was replicated six times.

Two months 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 spring 2008 at low, medium and high density, as specified in Table 2. Keeping the plots clean from other plants 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

**Table 2.** Number of *L. corniculatus* (L) 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)

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 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 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 1/2 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 optical emission spectroscopy (iCAP 6000 series, Thermo Scientific, Germany). The CCC samples were also analyzed for anion- and cation-resin extractable P using the method of Saggart 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.

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

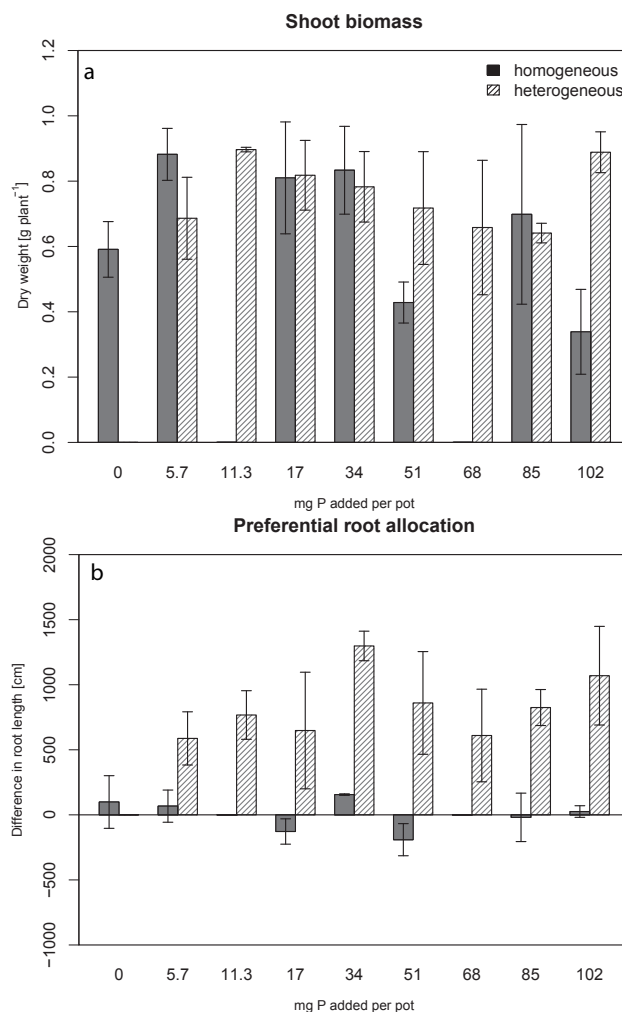
### 3.1 Climate chamber experiment

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. 2a).

In the heterogeneous treatments, root length was always significantly higher in the P-fertilized part of the containers than in the unfertilized part (95 % confidence interval  $> 0$ , see also Fig. 2b). 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

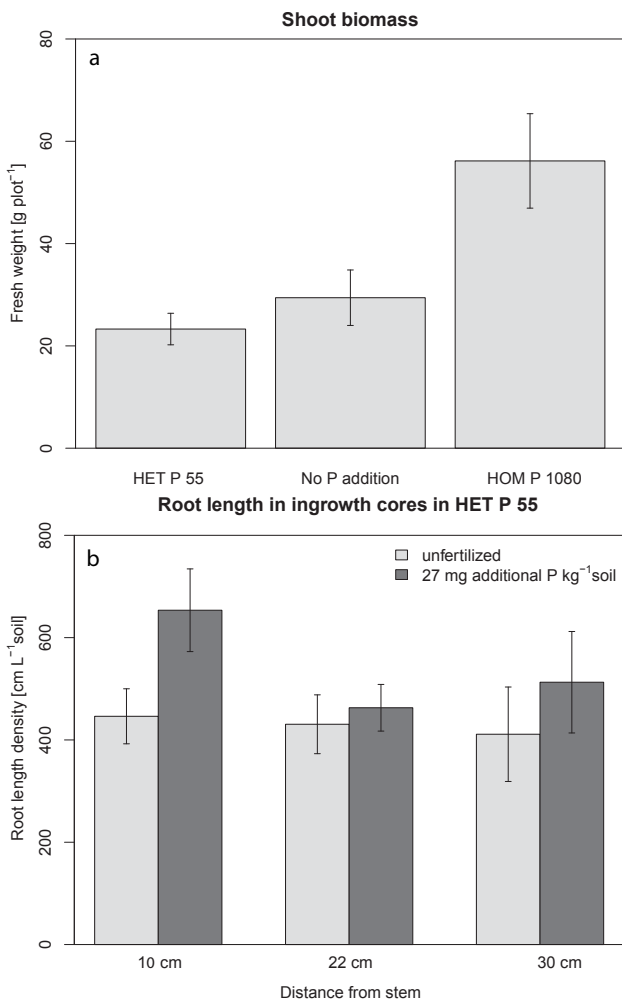
As we selected the plants according to their size, growth habitus and leaf shape for the ingrowth core field experiment, it can be assumed that they were genetically much more ho-



**Fig. 2.** (a) Shoot dry weight 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.

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 (HOM P 1080) treatment than in the ingrowth core (HET P 55) and control treatments (No P addition, Fig. 3a).

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. 3b 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



**Fig. 3.** (a) Shoot fresh weight production of single field-grown *L. corniculatus* plants grown on plots with heterogeneous P fertilization (HET P 55), no P addition (No P addition) or homogeneous P fertilization (HOM P 1080). (b) Root length density in fertilized and unfertilized ingrowth cores of HET P 55 at 10, 22 and 30 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,  $p < 0.05$ ).

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. 4). 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 be-

tween labile P and root length density on the CCC (Fig. 5). 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 the correlation between distance and water-soluble P was not significant. 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 also increased with distance on the CCC, but not on the ES.

Water-soluble Ca was on average  $28 \text{ mg kg}^{-1}$  soil higher in root-adjacent soil than in root-distant soil on the ES (Fig. 6), while the pH of root adjacent soil was on 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 CCC 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, similar 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* 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; Denton et al., 2006) 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. Furthermore, it indicates that the influence of the roots extended into zones around

**Table 3.** Pearson correlation coefficients (lower part of the table) and p-values (upper part of the table) for root length density, 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 the respective 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	<i>-0.14</i>	0.14	<i>-0.32</i>		<i>0.0283</i>
Water-sol. P	<i>-0.28</i>	<i>-0.05</i>	<i>-0.06</i>	<i>0.27</i>	

**Table 4.** Pearson correlation coefficients (lower part of the table) and p-values (upper part of the table) for root length density, 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 the respective variables ( $p > 0.05$ ).

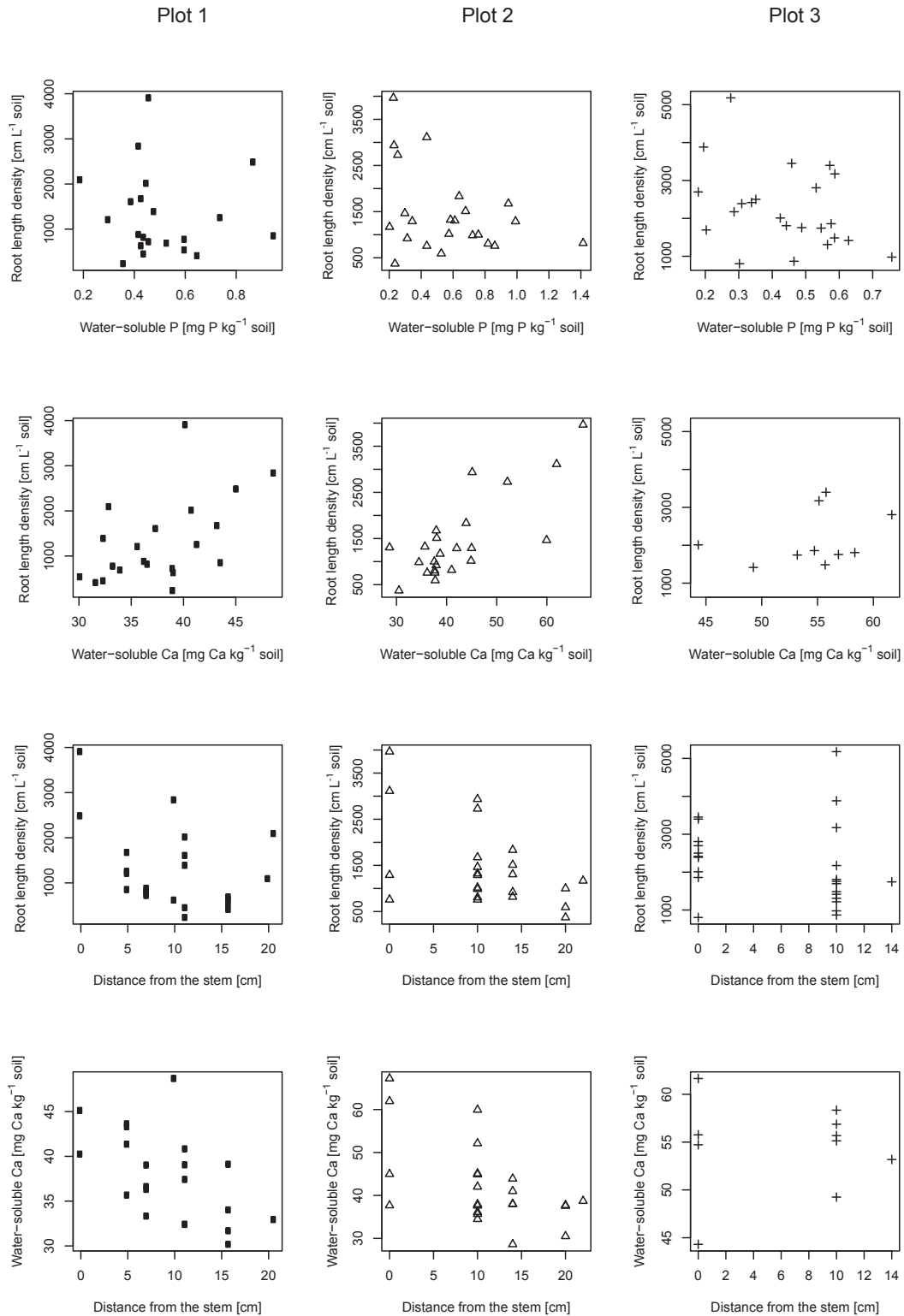
	Root length	Distance	Water-sol. Ca	pH	Water-sol. P	Labile P
Root length		0.1512	0.7251	0.4737	0.3016	<i>0.0038</i>
Distance	<i>-0.17</i>		<i>0.0372</i>	<i>0.0256</i>	0.1762	<i>0.0066</i>
Water-sol. Ca	<i>-0.04</i>	<i>-0.24</i>		<i>0.0004</i>	<i>0.0000</i>	0.0584
pH	0.08	<i>0.26</i>	<i>-0.40</i>		0.0783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

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 *Liquidambar styraciflua* and *Pinus taeda* monocultures. However, in contrast to our sites, these stands were already in a stage of ecosystem development at which P recycling with root and shoot litter decomposition was probably 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, while P release via necromass decomposition would be spatially much more concentrated, as it would occur in close relationship with the allocation of the mass of 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 on 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 soil 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

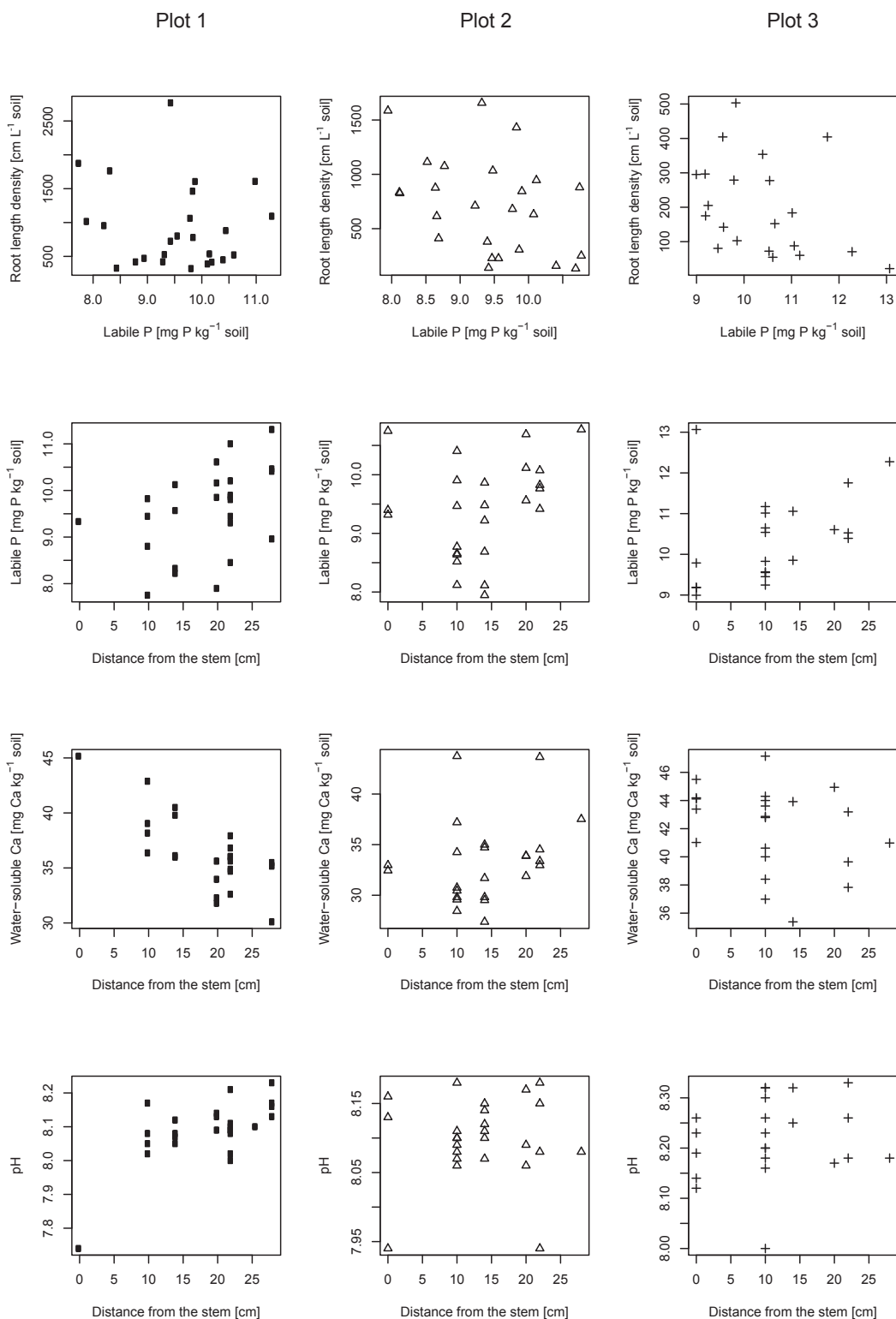
allocation in P-enriched soil zones. Several authors investigating root distributions in relation to soil nutrient distributions suggested that P heterogeneity in their study soils was too low to become relevant for root allocation in herbaceous plants, but not for trees (Farley and Fitter, 1999; Gallardo 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 overriding P uptake by roots (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

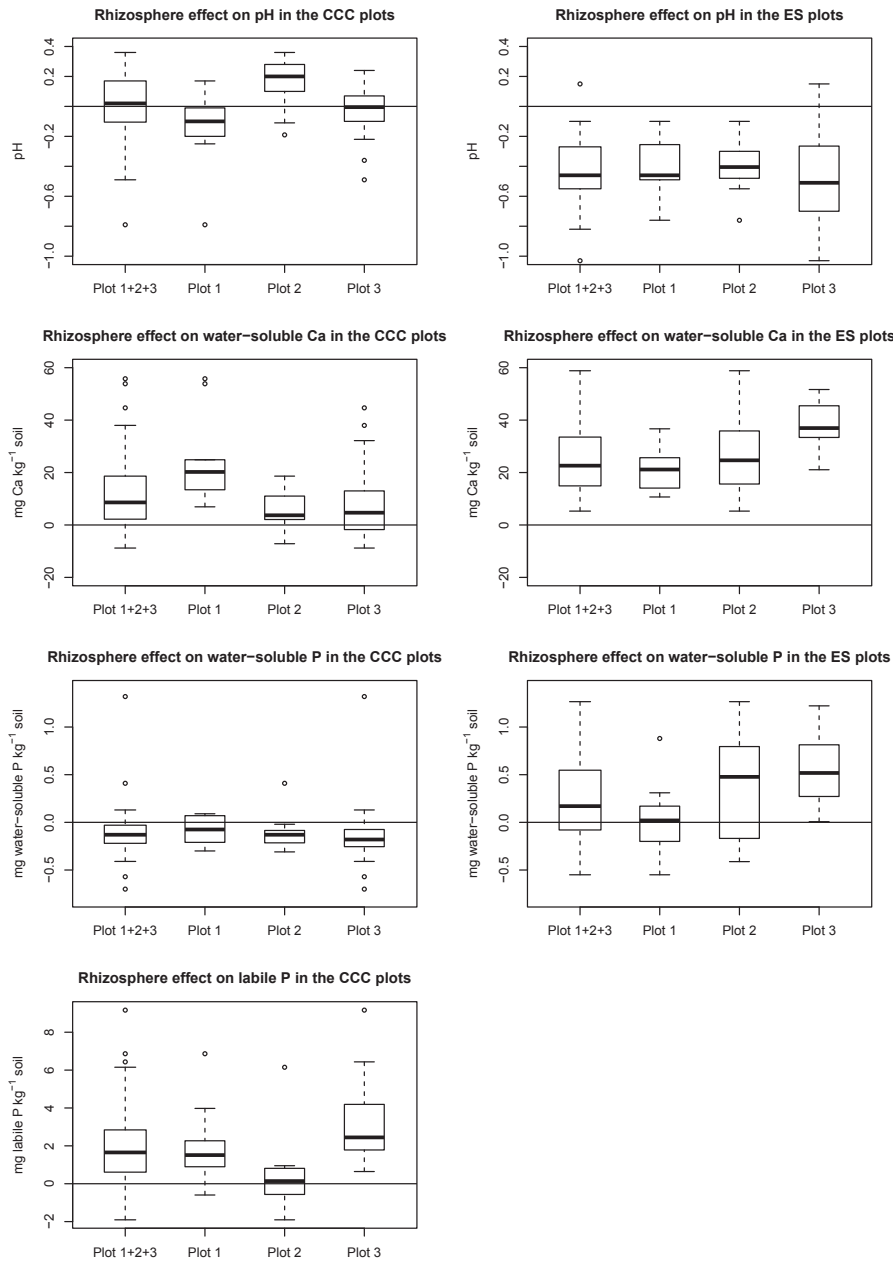


**Fig. 4.** The relationship between root length and water-soluble P (first row) or water-soluble Ca (second row) as well as the relationship between the distance from the stem and root length density (third row) or water-soluble Ca (fourth row) investigated in the high-density sampling on the experimental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





**Fig. 5.** The relationship between root length density 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 topsoil (0–10 cm) of the plots with low (plot 1), intermediate (plot 2) and high vegetation density (plot 3).



**Fig. 6.** 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.

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 explain why 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. 5). 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 negative correlation between stem distance and root length density was more pronounced in the one-year-old plants on the ES than in the plants of the CCC plots, which were on average older than one year. This indicates that with plant age new root growth is increasingly allocated at greater distances from the stem. This could be a response to nutrient depletion around older parts of the root system, as long as zones farther away are still more abundant in nutrients and

water. Indeed, we found a increase in 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.

The results suggest that patches with spontaneous P enrichment in the CCC and ES field soils that was due to natural spatial variability (i.e. heterogeneity that was not experimentally constructed) only persisted for less than a year, before they were depleted by root P uptake into patches. This would mean that also the pattern of new root growth allocation would shift accordingly during that time frame and with it the spatial pattern of root influences on the surrounding soil, including weathering, organic matter deposition and other processes affecting soil formation. Our study shows that the responsiveness of plant root allocation to nutrient enriched soil, as obviously found under well-controlled experimentally manipulated conditions, may not necessarily translate into a corresponding, easily interpretable relationship between root and nutrient distribution under undisturbed field conditions even at the early stage of the development of an ecosystem.

## 5 Conclusions

The experiments with heterogeneous and homogeneous P fertilization showed that P was a limiting factor for the growth of *L. corniculatus* on the experimental soil and that the plants preferentially allocated roots into P-enriched zones in this soil. The results of the high-density samplings, on the other hand, indicate that P depletion by roots (and probably mycorrhizal fungi) already had a more dominating influence on the spatial relationship between root length density and soil P concentrations in the field soil without artificially enhanced P heterogeneity within the first year after plant establishment. Assuming that *L. corniculatus* plants responded with preferential root allocation also to local P enrichment that was present in the undisturbed and untreated field soils due to natural spatial variability, this means that depletion of these patches by root P uptake subsequently turned them into their opposite, i.e. patches with decreased P availability. 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 heterogeneities, in particular locally concentrated P inputs with leaf and root litter.

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## References

- Buman, R. A., Schumacher, T. E., and Riedell, W. E.: A modified soil monolith technique for characterizing root systems, *Crop Sci.*, 34, 296–299, 1994.
- Caldwell, M. M., Manwaring, J. H., and Durham, S. L.: Species interactions at the level of fine roots in the field: Influence of soil nutrient heterogeneity and plant size, *Oecologia*, 106, 440–447, doi:10.1007/bf00329699, 1996.
- Denton, M. D., Sasse, C., Tibbett, M., and Ryan, M. H.: Root distributions of Australian herbaceous perennial legumes in response to phosphorus placement, *Funct. Plant Biol.*, 33, 1091–1102, doi:10.1071/fp06176, 2006.
- Eissenstat, D. M. and Caldwell, M. M.: Seasonal timing of root-growth in favorable microsites, *Ecology*, 69, 870–873, doi:10.2307/1941037, 1988.
- Farley, R. A. and Fitter, A. H.: Temporal and spatial variation in soil resources in a deciduous woodland, *J. Ecol.*, 87, 688–696, doi:10.1046/j.1365-2745.1999.00390.x, 1999.
- Gallardo, A. and Parama, R.: Spatial variability of soil elements in two plant communities of NW Spain, *Geoderma*, 139, 199–208, doi:10.1016/j.geoderma.2007.01.022, 2007.
- Gerwin, W., Schaaf, W., Biemelt, D., Fischer, A., Winter, S., and Huettl, R. F.: The artificial catchment “Chicken Creek” (Lusatia, Germany)-a landscape laboratory for interdisciplinary studies of initial ecosystem development, *Ecol. Eng.*, 35, 1786–1796, doi:10.1016/j.ecoleng.2009.09.003, 2009.
- Gross, K. L., Pregitzer, K. S., and Burton, A. J.: Spatial variation in nitrogen availability in 3 successional plant-communities, *J. Ecol.*, 83, 357–367, doi:10.2307/2261590, 1995.
- Hendriks, L., Claassen, N., and Jungk, A.: Phosphate-depletion at the soil-root interface and the phosphate-uptake of maize and rape, *Z. Pflanzen. Bodenk.*, 144, 486–499, doi:10.1002/jpln.19811440507, 1981.
- Hinsinger, P. and Gilkes, R. J.: Mobilization of phosphate from phosphate rock and alumina-sorbed phosphate by the roots of ryegrass and clover as related to rhizosphere pH, *Eur. J. Soil Sci.*, 47, 533–544, doi:10.1111/j.1365-2389.1996.tb01853.x, 1996.
- Hinsinger, P., Gobran, G. R., Gregory, P. J., and Wenzel, W. W.: Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes, *New Phytol.*, 168, 293–303, doi:10.1111/j.1469-8137.2005.01512.x, 2005.
- Hinsinger, P., Betencourt, E., Bernard, L., Brauman, A., Plassard, C., Shen, J. B., Tang, X. Y., and Zhang, F. S.: P for two, sharing a scarce resource: Soil phosphorus acquisition in the rhizosphere of intercropped species, *Plant Physiol.*, 156, 1078–1086, doi:10.1104/pp.111.175331, 2011a.
- Hinsinger, P., Brauman, A., Devau, N., Gerard, F., Jourdan, C., Laclau, J. P., Le Cadre, E., Jaillard, B., and Plassard, C.: Acquisition of phosphorus and other poorly mobile nutrients by roots. Where do plant nutrition models fail?, *Plant Soil*, 348, 29–61, doi:10.1007/s11104-011-0903-y, 2011b.
- Hubel, F. and Beck, E.: In-situ determination of the P-relations around the primary root of maize with respect to inorganic and phytate-P, *Plant Soil*, 157, 1–9, 1993.
- Huetsch, B. W., Augustin, J., and Merbach, W.: Plant rhizodeposition: An important source for carbon turnover in soils, *J. Plant Nutr. Soil Sci.*, 165, 397–407, 2002.
- Jackson, R. B. and Caldwell, M. M.: Geostatistical patterns of soil heterogeneity around individual perennial plants, *J. Ecol.*, 81, 683–692, 1993.
- Jansa, J., Mozafar, A., and Frossard, E.: Phosphorus acquisition strategies within arbuscular mycorrhizal fungal community of a single field site, *Plant Soil*, 276, 163–176, doi:10.1007/s11104-005-4274-0, 2005.
- Kume, T., Sekiya, N., and Yano, K.: Heterogeneity in spatial P-distribution and foraging capability by *Zea mays*: Effects of patch size and barriers to restrict root proliferation within a patch, *Ann. Bot.-London*, 98, 1271–1277, doi:10.1093/aob/mcl216, 2006.
- Ma, Q. and Rengel, Z.: Phosphorus acquisition and wheat growth are influenced by shoot phosphorus status and soil phosphorus distribution in a split-root system, *J. Plant Nutr. Soil Sci.*, 171, 266–271, doi:10.1002/jpln.200700183, 2008.
- Ma, Q. F., Rengel, Z., and Bowden, B.: Heterogeneous distribution of phosphorus and potassium in soil influences wheat growth and nutrient uptake, *Plant Soil*, 291, 301–309, doi:10.1007/s11104-007-9197-5, 2007.
- Majdi, H., Smucker, A. J. M., and Persson, H.: A comparison between minirhizotron and monolith sampling methods for measuring root-growth of maize (*Zea mays* L.), *Plant Soil*, 147, 127–134, doi:10.1007/bf00009378, 1992.
- Meiwes, K. J., König, N., Khanna, P. K., Pretzel, L., and Ulrich, B.: Chemische Untersuchungsverfahren für Mineralboden, Auflagehumus und Wurzeln, Ber. D. Forschungszentrums, Waldökosysteme, 7, 1984.
- Milchunas, D. G., Lee, C. A., Lauenroth, W. K., and Coffin, D. P.: A comparison of C-14, Rb-86, and total excavation for determination of root distributions of individual plants, *Plant Soil*, 144, 125–132, doi:10.1007/bf00018853, 1992.
- Millikin, C. S. and Bledsoe, C. S.: Biomass and distribution of fine and coarse roots from blue oak (*quercus douglasii*) trees in the northern Sierra Nevada foothills of California, *Plant Soil*, 214, 27–38, 1999.
- Mou, P., Jones, R. H., Mitchell, R. J., and Zutter, B.: Spatial-distribution of roots in sweetgum and loblolly-pine monocultures and relations with aboveground biomass and soil nutrients, *Funct. Ecol.*, 9, 689–699, doi:10.2307/2390162, 1995.
- Robinson, D.: The responses of plants to nonuniform supplies of nutrients, *New Phytol.*, 127, 635–674, 1994.
- Saggar, S., Hedley, M. J., and White, R. E.: A simplified resin membrane technique for extracting phosphorus from soils, *Fert. Res.*, 24, 173–180, 1990.
- Schlichting, E., Blume, H. P., and Stahr, K.: *Bodenkundliches Praktikum*, 2. Auflage, Blackwell, 1995.
- Smith, S. E., Smith, F. A., and Jakobsen, I.: Functional diversity in arbuscular mycorrhizal (AM) symbioses: The contribution of the

- mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake, *New Phytol.*, 162, 511–524, doi:10.1111/j.1469-8137.2004.01039.x, 2004.
- Sprent, J. I., Becana, M., and Sutherland, J. M.: Optimizing nitrogen fixation in legume crops and trees, edited by: Bothe, H., De Bruijn, F. J., and Newton, W. E., Gustav Fischer Verlag, 725–734, 1988.
- Van Veldhoven, P. P. and Mannaerts, G. P.: Inorganic and organic phosphate measurements in the nanomolar range, *Anal. Biochem.*, 161, 45–48, 1987.
- Vitousek, P. M., Porder, S., Houlton, B. Z., and Chadwick, O. A.: Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen-phosphorus interactions, *Ecol. Appl.*, 20, 5–15, doi:10.1890/08-0127.1, 2010.
- Wang, Z. Y., Kelly, J. M., and Kovar, J. L.: Depletion of macronutrients from rhizosphere soil solution by juvenile corn, cottonwood, and switchgrass plants, *Plant Soil*, 270, 213–221, doi:10.1007/s11104-004-1538-z, 2005.
- Weligama, C., Tang, C., Sale, P. W. G., Conyers, M. K., and Liu, D. L.: Localised nitrate and phosphate application enhances root proliferation by wheat and maximises rhizosphere alkalisation in acid subsoil, 2nd International Conference on Rhizosphere, Montpellier, France, ISI:000259742700010, 101–115, 2007.