CHARACTERIZATION OF ROOT GROWTH DYNAMICS IN THE INITIAL PHASE OF SOIL DEVELOPMENT – LINKING 3D SAMPLING AND MINIRHIZOTRONE OBSERVATIONS

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Summary

Information about root architecture, root growth dynamics of *Lotus corniculatus* and *Calamagrostis epigeios*, the physical and chemical status of the bulk and rhizospheric soil have been gathered to understand how soil conditions influence root growth and roots, and, in return, how the chemical and physical status of soils influences the soil development in the initial phase of ecosystem development. Present findings indicate that the spatial distribution pattern of root systems is highly variable and is determined by both soil physical conditions and the genotype influencing the morphology of root proliferation, namely root branching. Both plants have a similar root/shoot ratio when calculated on a biomass basis. However, the root surface area and the total leaf area of *Lotus* were clearly higher than those of *Calamagrostis.* The spatial distribution of roots (monolith method) shows, that *Lotus* develops a rather shallow root system with a major amount of roots in 0 – 20 cm soil depth whereas roots of *Calamagrostis* tend to proliferate into deeper soil layers. Observations in minirhizotrones showed pronounced differences between *Lotus* and *Calamagrostis* with regard to root growth dynamics and vertical root proliferation. On the other hand results gave clear evidence that the physiological activity of roots has a strong impact on nutrient depletion and acidification particularly at the soil root interface. The alteration of the pH in the bulk soil and in the rhizosphere soil was much stronger for the legume than for the grass species indicating that nutrient uptake was much pronounced for *Lotus corniculatus*. Present results emphasize the benefit and need for having data from root growth dynamics, spatial distribution of roots and from chemical and physical soil conditions to be considered for a sound modelling of root growth.

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1   INTRODUCTION

To characterize the role of root growth for soil development in the initial state of ecosystem development root systems of the primary vegetation growing on raw soils from quaternary calcareous sediments was studied. Two abundant plants species, *Lotus corniculatus* (Gewöhnlicher Hornklee) a dicotyle flowering plant belongs to family of Fabaceae (legumes) and *Calamagrostis epigeios* (Land-Reitgras) a genus in the herbaceous family Poaceae were selected and studied in detail under field conditions. A 3-dimensional root sampling procedure and observations from minirhizotron were used to link information on root distribution at high resolution of data on growth dynamics obtained from minirhizotrone observations for same pioneer plant species. The present study is focussing on the active response of root growth to heterogeneously distributed nutrient pools in soils, and will in reverse characterize the effects that root proliferation may have on the allocation of nutrients. This will provide information to what degree roots may actively change their chemical environment and how the chemical status of a soil in the initial state of soil development may drive the spatial distribution of roots. Linking data from 3D root sampling with those of the seasonal root growth dynamics will provide a unique chance to develop a dynamic root growth model for plant species prevailing in the primary state of succession. As such the model will integrate the effects caused by both plant species and soil conditions and will strive for validate and improving current soil hydrological models. At the same time it will support the assessment of root derived carbon and nutrient allocation in soils. From this perspective the linking of information from 3D root distribution and minirhizotrone studies is supposed to open up new opportunities to explore the role of roots for soil development.

2   MATERIAL AND METHODS

Two abundant plants species, *Lotus corniculatus* (L) and *Calamagrostis epigeios* (C) were chosen and studied in detail in the initial stage of ecosystem development stage under field conditions using the information from minirhizotrone tubes and from three dimensional (3D) high resolution roots sampling in a complementary way. Whereas images from minirhizotrone tubes give an insight into root growth dynamics and turnover, 3D sampling of roots delivers spatially explicit volume related information on root distribution and gives access to bulk and rhizosphere soil. Minirhizotrone tubes (length: 200 cm; intern. diameter 5,54 cm) were fixed across a 18 m^3 meter soil pit at 400 x 200 x 200cm soil depth with a space of 30cm between the tubes. After the instalment the soil pit was filled with quaternary calcareous sediments compact the soil layer wise through irrigation of water, and hence facilitating a tight closure between tubes and the surrounding soil. On top of the minirhizotrone complex 4 month old seedling of *Lotus corniculatus* and *Calamagrostis epigeios* were planted which had been grown and cultivated from seeds under greenhouse conditions under natural light conditions and low nutrient supply. Plants for sampling of monoliths were sowed on same substrates adjacent to the minirhizotrone complex. High resolution 3D root sampling of monoliths was carried out for two years old seedlings.

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Three soil monoliths (50 x 50 x 50 cm) were chosen for the 3D sampling representing different vegetation densities (number of shoots). Sampling was carried out from late April – to early July 2009. The monoliths were sampled layer wise (0 – 10 cm, 10 – 20 cm, 20 – 30 cm, 30 – 40 cm, 40 – 50 cm) with 25 samples per each layer. Samples were put into plastic bags and immediately stored in thermo boxes and subsequently in the laboratory refrigerator at approx. 4°C. Root sampling and root preparation were carried out parallel along the sampling period. Roots were separated from the bulk soil after an initial sieving of samples followed by a thorough collection of roots. Soil adhering to the roots was gently brushed from roots and kept apart from bulk soil samples from a subsequent chemical analysis. After harvesting of rhizosphere soil, roots were cautiously washed by continuous flotation of water. Root surface area, number of root branches and the root length density were calculated for moist root samples using an Epson perfection V700 scanner at 400dpi resolution and WinRHIZO software (Regent Instruments, Inc. Quebec Canada, version 2009a) for digitalization and further processing of the images. For the determination of weights of biomass, roots and shoots were dried at 65°C for 3 days. The present paper will show first results of the soil analysis with regard to pH values in water extracts (1 : 2,5 w:v, soil : water) and water soluble nutrient contents for bulk soil, exemplified phosphor.. Soil texture was determined using method of DIN ISO 11377 [9]. The weekly measurement of soil humidity (Vol %) were carried out by using a Profile sample type PR2 (Delta-T Devices LtD, Cambridge, UK).The statistical analyses of data comprised mean, standard deviation, Mann-Whitney-U-test, t-test, correlation co-efficient after Pearson and were performed using SPSS module bivariate correlation and comparison of means.

3 RESULTS AND DISCUSSION

3.1 Soil conditions

soil depth [cm]	Bulk soil density	soil depth [cm]	coarse sand [%]	medium sand	fine sand	sand total	coarse clay	clay
0	$1,51 \pm 0,08$	0 - 30	8,5	55,17	32,63	96,3	1,6	2
10	$1,64 \pm 0,02$							
30	$1,64 \pm 0,01$	30 - 60	8,5	55,17	32,63	96,3	1,6	2
60	$1,56 \pm 0,01$							
100	$1,57 \pm 0,03$							

Table.1 : Soil texture and bulk soil density of substrates taken from the minirhizotrone grid.

At the experimental sites sandy substrates were composed of mainly medium (55.2 %) and fine (32.6 %) sand (Tab.1) lacking any soil organic carbon. The share of coarse clay and clay amounted to 3.2 % indicating an extremely low water storage capacity of the prevailing substrate. In the initial phase the volumetric water contents ranged from 5 to 18 % with lowest values for the topsoil and highest for the deeper soil layers (data not shown). For this observation period no severe water scarcity could be detected [1]. The bulk soil density showed little differences between top and deep soil layers indicating that the soil physical

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conditions were quite homogenous and the substrate had not been composed to any compaction which might have interfered with root proliferation.

3.2   Growth parameter

	L. corniculatus	C. epigeios
total leaf area [cm²]	2214	1271
total root surface area [cm²]	8050	6272
shoot/root ratio [rel. units]	0,87	0,81
specific. leaf area [cm² g^{-1}]	179	136
total leaf area/ total root surface area [rel. units]	0,28	0,20

Table 2 : Growth parameters for the vegetation of two year old *Lotus corniculatus* and *Calamagrostis epigeios* *plants* per soil monolith.

A   B
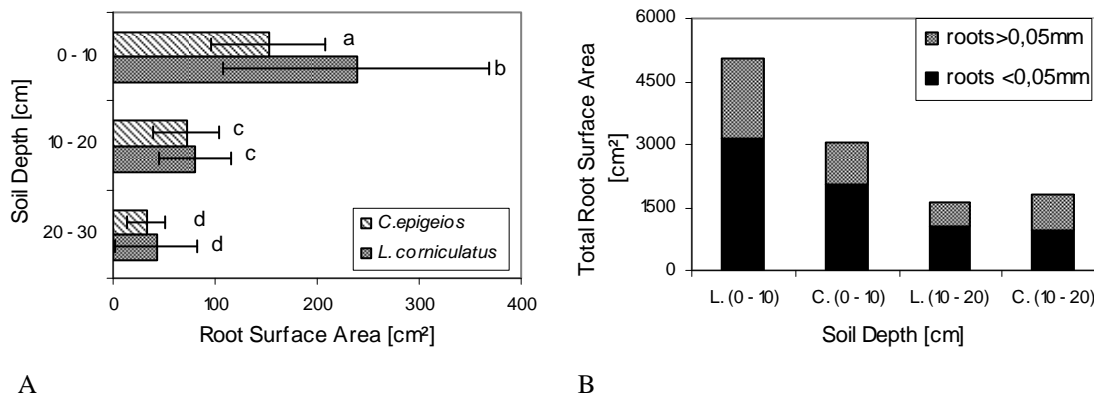
Figure 2A : Comparison of root surface area for *Calamgrostis epigeios* and *Lotus corniculatus* in different soil depths from 3D sampling of soil monoliths. 2B : Comparison total parts of roots < 0,05mm diameter with total part of roots with diameter > 0,5mm relating to total root surface area for *Lotus corniculatus* (L) and *Calamagrostis epigeios* (C). n = 5 – 25

Competition between shoots and roots for photosynthates plays an important role for the survival strategy of species that settle in the initial phase of succession and appears to be ruled by the specific genotype. However, the relationship between shoot and root growth (shoot-root ratio) may be strongly modified by external factors such as nutrient- and water availability supply, light conditions, heterogeneity of soil physical conditions and may even change during ontogenesis [2]. The root/ shoot ratio was calculated on a biomass basis using data from the high resolution 3D sampling of soil monoliths. They turned out to be nearly the same for both plant species under investigation at the same ontogenetic age and with equal number of shoots per monolith. That may lead to the assumption that both species allocate their assimilates to roots and shoots in a similar way (Tab.2). However, taking into consideration the specific leaf area index and the total root surface area (RSA) as the physiologically relevant parameters pronounced differences can be observed between *Lotus corniculatus* and *Calamgrostis epigeios* since the total leaf area was almost 70 % and root surface area 28 % higher for *Lotus* than for *Calamagrostis*. Correspondingly a significantly

higher ratio of total leaf area to total roots surface area was found for *Lotus* (Tab. 2) indicating a higher relative assimilation area of *Lotus corniculatus* and, hence, a higher photosynthetic rate. Differences in RSA were most pronounced in the top soil layer (0 – 10 cm; Fig. 2A) whereas no such differences in RSA between these two plant species were found in deeper soil layers. After partitioning the roots into two categories (>0,05mm-coarse roots; <0,05mm-fine roots), the percentage of fine roots in relation to root surface area was higher for coarse than for fine roots for both plant species. For modelling water and nutrient uptake a full assessment of root parameter such as root length density (cm cm^{-3}) is indispensable [3, 4]. Root length density (cm cm^{-3}) was nearly the same for *Lotus* and *Calamagrostis* (Fig.3) indicating a similar impact on water and nutrient uptake. However, present findings gave evidence that the retention of nutrients in 0 – 10 cm soil depth was much more pronounced for *Lotus* than for *Calamagrostis* (data not shown). From this result it can be concluded that besides root architecture the physiological influence of roots on the nutrient and certainly on the water cycling as well is strongly dependent on the specific plant species.
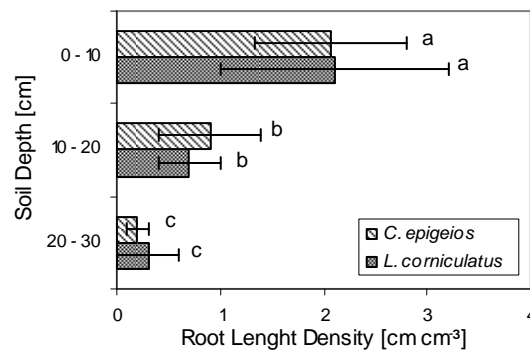

Figure 3 : Comparison of Root Length Density from *Calamgrostis epigeios* and *Lotus corniculatus* in different soil depths by monolith method. n = 5 – 25

3.3   Three dimensional root distribution

In order to compare the three dimensional (3D) structure of roots systems for both species we collected samples from 3 monoliths for each plant species considering differences in numbers of shoots per plant species. Figure 4 shows the 3D diagrams of root surface area for both plants. Root surface area per volume soil in the topsoil layer (0 – 10 cm) was significantly higher for *L. corniculatus* plants than for *C. epigeios* for all vegetation densities. For *L. corniculatus* no close relation could be observed in the topsoil layer between the number of shoots and RSA whereas RSA of *Calamagrostis* was closely linked to the number of shoots (Fig. 4). At the same time root proliferation turns out to increase for *C. epigeios* much stronger than for *L. corniculatus* in deeper soil layers showing that the root system of *Lotus* as legume tends to develop a rather shallow root system, whereas the root system of *C. epigeios* proliferates into the deeper soil. Therefore it is concluded that the prevalence of *Lotus* in the initial stage of succession conflicts with frequent drought events. In contrast *C. epigeios* appears to be much more adapted to water scarcity based on deeper rooting system. Surprisingly, deep soil penetration for both *L. corniculatus* and *C. epigeios* was much higher

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in the minirhozotron grid than in soil monoliths which highlight the role of bulk soil density for root proliferation [5] particularly in deeper soil layers. In figure 5 root growth dynamics as calculated from minirhizotron observations are shown. Pronounced differences could be observed between *L. corniculatus* and *C. epigeios* with regard to both root growth dynamics and vertical proliferation of the root system. 6 months after planting the seedlings roots from both species could be observed even in 200 cm soil depth. Root densities were much higher then for *L. corniculatus* than for *C. epigeios*. A major increase of RSA could be observed in May and July which was most pronounced for *C. epigeios* in the topsoil layer (0 - 10 cm) whereas root surface area of *L. corniculatus* began to slightly decrease at the same time. In contrast the root surface area of *C. epigeios* below 70 cm soil depth decreased towards the end of the vegetation period and turned out to be less abundant. At the same time a significant increase of RSA could be observed in the deeper soil for *L. corniculatus* roots (Fig. 5). The present finding makes evident that the genetic influence on root system morphology may be altered by soil physical conditions which may have a significant impact on interception and the preferential flow of water in the root zone.
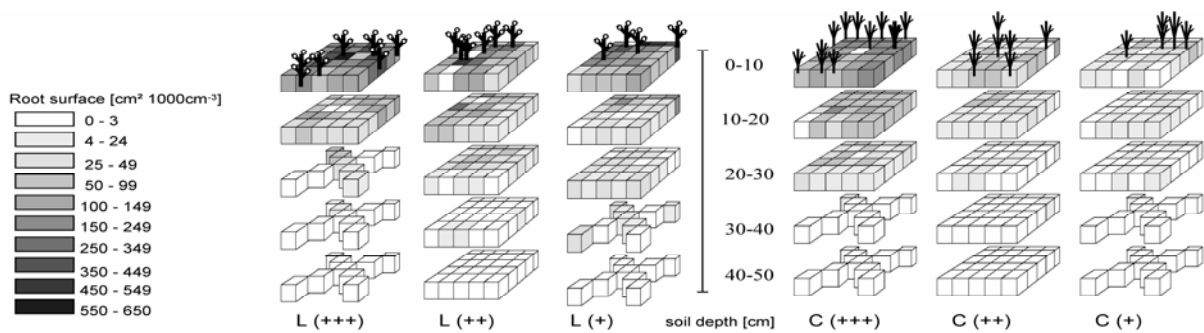

Figure 4 : Three dimensional image of root distributions based on root surface area (cm² 1000cm⁻³). For *Lotus corniclulatus* (L) and *Calamagrostis epigeios* (C) considering high (+++), middle (++) and low (+) vegetation density.
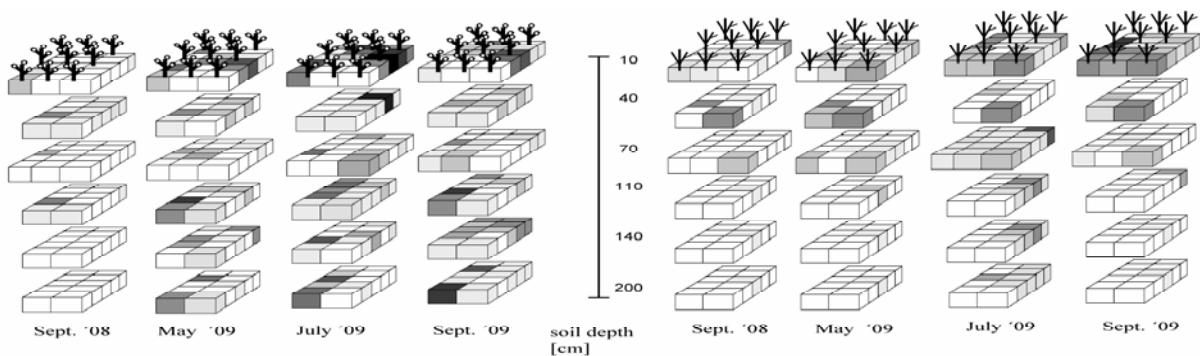

Figure 5 : Three dimensional image of root distribution from minirhizotrone observations in September, 2008 and in May, July, and Sept 2009.

3.4   pH Analysis

vegetation	soil depth [cm]	pH bulk soil [rel. Units]	pH rhizosphere soil [rel. Units]	ΔpH
without plants	0 - 10	$8,50 \pm 0,1$ [a]	/	/
	10 - 20	$8,50 \pm 0,1$ [a]	/	/
L. corniculatus	0 - 10	$8,14 \pm 0,13$ [b]	$7,60 \pm 0,26$ [a]	$0,54 \pm 0,20$ [a]
	10 - 20	$8,52 \pm 0,04$ [a]	$8,18 \pm 0,18$ [b]	$0,35 \pm 0,20$ [b]
C. epigeios	0 - 10	$8,32 \pm 0,07$ [c]	$8,12 \pm 0,18$ [b]	$0,20 \pm 0,15$ [b]
	10 - 20	$8,44 \pm 0,06$ [d]	$8,22 \pm 0,08$ [b]	$0,22 \pm 0,10$ [b]

Table 3 : pH data from bulk soil, rhizosphere soil and difference between pH of bulk – and associated rhizosphere pH (ΔpH). Means (±) are given in data. Within variables, measures with different superscripts are significantly different at $P \leq 0.01$. n = 11

Physical and chemical conditions may rule the vitality and proliferation of roots. In return, roots are able to change their physical and chemical environment. In table 3 the average pH values from rhizospheric and associated bulk soil samples are shown for monoliths covered by *L. corniculatus* and *C. epigeios* and for adjacent monoliths without vegetation. The average soil pH was highest for substrates without plant coverage. Significant differences were also found between species with higher values for *C. epigeios* than for *L. corniculatus* which was most distinct in the top layer (0 - 10 cm). Lowest soil pH was detected in the rhizosphere with a most pronounced gradient for *L. corniculatus*. These results indicate that the release of H^+, HCO_3^-, in the course of nutrient uptake and the excretion of exudates [6] may significantly contribute to the acidification of both the rhizospheric and the bulk soil. Nitrogen plays a most prominent role for cation/anion uptake and thus for rhizosphere pH. The cation uptake of plants living in symbiosis with N_2 fixing bacteria is ruled by ammonium [6, 7]. The uptake of ammonium induces a high release of H^+ in the rhizosphere. Furthermore, 30 – 60 % of N_2- fixation energy releases *Rhizobia* in form of H_2 [8]. This may well explain why the bulk soil pH was much lower for *L. corniculatus* than for *C. epigeios* and why differences between rhizosphere and bulk soil pH were highest for *L. corniculatus*.

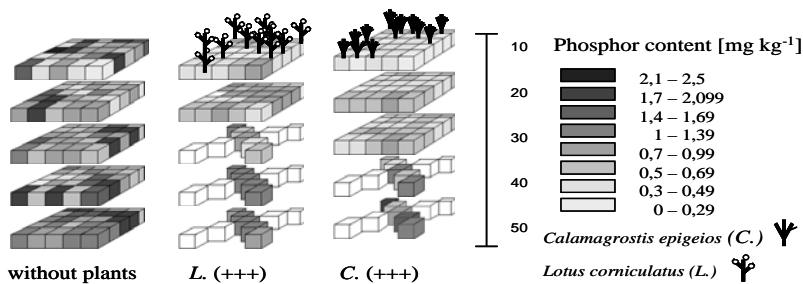## 3.5 Three dimensional phosphorus distribution

Figure 6 : Three dimensional image of water soluble phosphor contents (mg kg^{-1}) for *Lotus cornicluatus* (L) and *Calamagrostis epigeios* (C) considering high (+++) vegetation density.

In Figure 6 the 3 dimensional distribution of water soluble phosphor in tree monoliths is shown. The phosphorus distribution was very heterogeneous in monoliths without plants. Pronounced differences could be observed in monoliths of both plant species. Clearly lower phosphorus content was detected in bulk soil layers 0 – 30 cm, with lowest contents in 0 – 10 cm. Bulk soil of *C. epigeios* showed explicit lower phosphorus contents as those of *L. corniculatus*. Moreover there was acutely homogenous phosphorus distribution in overgrown monoliths with *L. corniculatus* some more in *C. epigeios*. These results indicate that the presence of roots from diverse plants have a different influence on changes of nutrient contents, distribution and certainly on the water cycling in soil.

4   ACKNOWLEDGEMENTS

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