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Root Growth of *Hordeum vulgare* and *Vicia faba* in the Biopore Sheath

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Abstract: Biopores provide nutrients from root debris and earthworm casts. Inside large biopores, root function is limited due to the lack of root–soil contact. However, the immediate surroundings of biopores may hold a key function as "hotspots" for root growth in the subsoil. To date, sufficient quantitative information on the distribution of roots and nutrients around biopores is missing. In this field study, the biopore sheath was sampled at distances of 0–2, 2–4, 4–8, and 8–12 mm from the surface of the pore wall. The results show a laterally decreasing gradient from the pore towards 8–12 mm distance in root length density (RLD) of spring barley (*Hordeum vulgare* L.) and faba bean (*Vicia faba* L.), as well as in total nitrogen (N_t)- and total carbon (C_t)-content. In the biopore sheath (2–12 mm), the share of roots with a diameter of less than 0.4 mm was 92% for barley and 89% for faba bean. The findings support the view that roots can utilize biopores to gain access to deeper soil layers and may use the sheath for nutrient uptake and entrance through to the bulk soil. However, especially for barley, the inner layer of the biopore sheath appeared to be more important for root growth than the sheath of farer distance.

Keywords: biopore sheath; root architecture; root diameter; root distribution; root length density; subsoil

1. Introduction

Nutrient acquisition from subsoil can vary significantly, as it is influenced by climate, soil type, resource availability, soil compaction, and root architecture [1–3]. Generally, the subsoil becomes more important when topsoil is dry [4] or nutrient-depleted (e.g., [5,6]). In low-input farming systems, such as organic agriculture, it is generally aimed to avoid large amounts of inputs and to manage farms according to the principle of nearly-closed nutrient cycles. For organic agriculture, the utilization of subsoil resources is immanent. Physical access into deeper soil layers is easily possible through biopores, because of less mechanical impedance [7]. Biopores are known as "hotspots" for biological activity and root growth in heterogeneous subsoil [8,9]. This is due to their favorable properties, such as (i) higher gas and air exchange, (ii) nutrient enrichment, due to organic material originating from earthworm casts and decayed roots, and therefore (iii) higher microbiological activity (e.g., [10,11]). This study focuses on the biopore sheath of continuous macropores with diameters >5 mm, which are

formed by plant roots and anecic earthworms. In untilled soil layers, they can exist from several years to decades [12]. The ratio of root length inside biopores over the total root length within soil horizon under study has shown considerable variety in previous studies (Table 1). The ratios are ranging from 21%, <25%, and 11–26% in biopore lumen, up to 80% in biopore lumen and 1 mm sheath, or between 44 and 95% (increasing share with increasing soil depth). In studies on biopores, there is a lack of the quantitative information about root growth throughout the sheath. That is, however, important, because roots should be in physical contact with solid soil particles in order to mobilize and take up nutrients.

Table 1. The share of roots in biopores over the total root length.

Soil Type	Crop	Share of Roots [%]	Reference
Haplic Luvisol (silty loam)	Winter barley, Oilseed rape	21	Perkons et al. [13]
Haplic Luvisol (silty loam)	Winter barley	<25	Kautz et al. [14]
Black Vertosol	Pasture, dominated by Queens-land blue grass and Tall oat grass	11–26	Stewart et al. [15]
Typic and Haplic Palexeralf (hard setting clay)	Wheat	80 *	Pierret et al. [16]
Red Kondosol (acidic loam)	Wheat	44–95 **	White and Kirkegaard [17]

* Share of roots in biopore lumen and 1 mm biopore sheath. ** Increasing share with increasing soil depth.

In smaller biopores with diameters a little larger than root diameter it is easier for roots to establish contact to the solid soil phase. In contrast, in larger sized biopores (e.g., >5 mm), where pore diameter is multiple times greater than root diameter, root–soil contact can eventually be lost. For instance, Stirzaker et al. [18] found a poor root–soil contact in biopores, and Passioura [19] reported about clumping roots in macropores with a poor root–soil contact, which presumably hindered water uptake and resulted in reduced leaf area. Athmann et al. [20] found, using in situ endoscopy, that 85% of barley and oilseed rape roots established root–soil contact in biopores at the biopore wall, or crossing the biopore lumen. However, at least some portions of these roots that were counted as "in contact" were still exposed to air. Therefore, roots, which are growing into the biopore sheath are in closer contact to an area with enriched nutrients. Especially, the walls of earthworm burrows (defined as drilosphere) have been found to be enriched with carbon, nitrogen ([21] reviewed by [22]), [23], and macronutrients [24], as well as with microorganisms [25]. There are different results on the extension of the nutrient enrichment, ranging from a distance from macropore of 2 mm ([21] reviewed by [22]), 3 mm [24], 3.5 mm [26], 4 mm [27,28], up to 8 mm [29]. Furthermore, Andriuzzi et al. [29] observed, in a millimeter-scaled sampling, that the enrichment of ^{13}C and ^{15}N (with labeled earthworm fodder) in the immediate surroundings of earthworm channels in a grassland-topsoil was laterally decreasing until 8 mm distance from macropore. It is uncertain if the nutrient enrichment is attributed to an increased root growth within the biopore sheath.

In heterogeneous subsoil, roots grow through different voids, pores, and fissures of different sizes [30]. Ehlers et al. [7] showed that roots can bypass compacted soil layers by growing within biopores and using them as a preferential pathway into deeper soil layers. Physical properties of biopore formation by roots, as described by Uteau et al. [31], facilitate an increased air and water circulation within those deeper layers. Therefore, a well-established pore network may have positive effects on root growth. However, the surroundings of biopores are assumed to be compacted, owing to earthworm activities [32] and radial root growth [33,34]. Schrader et al. [35] observed compaction in the surroundings with a homogenous dense arrangement of soil particles. They also found a higher bulk density up to 2.2 cm radius, as a concentric area around the macropore, compared to bulk soil [35]. The displacement of soil particles during the process of pore formation may compact the surroundings. Additionally, Pagenkemper et al. [36] found that earthworm activity, especially linings

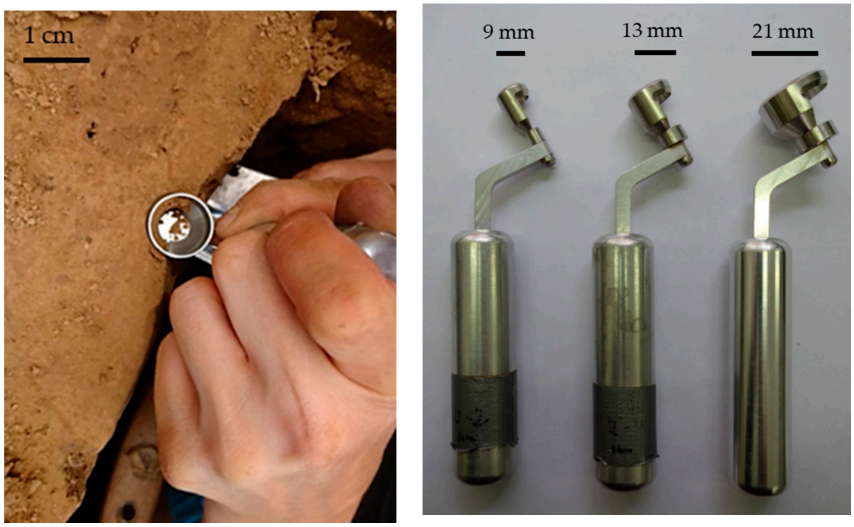
of earthworm casts, disconnect lateral pores and therefore might prevent entrance to the sheath and bulk soil. Further on, White and Kirkegaard [17] observed clumping roots of wheat in biopores in a hard setting soil, and Kutschera et al. [2] drew clustered roots, laterals, and/or root hairs of spring barley in biopores in a dense soil. By these findings, the roots seemed to be trapped within the biopore lumen. Thus, the sheath might be obstructive for the entrance of roots to the bulk soil, since high mechanical resistance, especially in the axial direction, hinders root elongation [37,38]. Nevertheless, Athmann et al. [20] observed roots laterally entering and leaving the biopore lumen, and results by Perkons et al. [13] showed higher root length densities (RLD) in biopores, as well as in bulk soil with increasing biopore density. Therefore, it can be suggested that roots are able to leave the biopore lumen and enter the biopore sheath.

According to these results, the spatial extension of the biopore sheath is varying, depending on its biological, chemical and physical characteristics. Referred to root growth, the biopore sheath has been defined as the surrounding soil in direct vicinity to the pore, which contains 80% of the roots [15]. However, there is insufficient evidence as to what extent root proliferation into the biopore sheath occurs. Roots growing through the surroundings of biopores can establish root–soil contact easily and draw benefit from the high concentration of nutrients, microbiological activity, and easier access to water and oxygen. Thus, it is necessary to investigate root distribution in the biopore sheath of biopores in subsoil. In this study, the biopore sheath includes lateral distances from macropore until 12 mm. The hypothesis of this research is that (i) RLD and (ii) N_t- and C_t-content decrease from pore towards 8–12 mm lateral distance, and (iii) the proportion of fine and small roots is higher in the sheath than in the biopore lumen.

2. Materials and Methods

2.1. Experimental Setup

The study was performed at a field trial in Klein-Altendorf near Bonn, Germany (50°37′8.5″ N, 6°59′25.4″ E, 150 m.a.s.l.) with 625 mm annual precipitation and a mean annual temperature of 9.6 °C. The soil is classified as a Haplic Luvisol, which has developed from loess (details in [39]). The root distribution of spring barley (*Hordeum vulgare* L. "Barke") and faba bean (*Vicia faba* L. "Fanfare") was observed. The precrops were 2017: oat (*Avena sativa* L.), followed by chicory (*Cichorium intybus* L.) as a catch crop, 2016: winter barley (*H. vulgare* L.), 2015: spring oilseed rape (*Brassica napus* L.), and 2014: chicory (*C. intybus* L.). For each species, two plots were established. The plot size was 4 × 2 m, and eight biopores per plot were investigated. Sampling took place between 22 May and 3 June 2018, right before, and at the flowering. Crop biomass (*H. vulgare* L. DC 52–55, *V. faba* L. DC 63–65, BBCH-scale) was sampled for analysis of the nutrient state of N, P, and K. In order to sample the biopores, profile trenches were arranged. Samples were taken from the Bt-horizon in 45 to 105 cm soil depth. The bulk density in this horizon was 1.42 g cm^{-3} in 41–75 cm and 1.52 g cm^{-3} in 75–115 cm soil depth in a reference soil profile [39]. First, topsoil was removed down to a depth of 45 cm. From the core plot, eight biopores with a diameter of at least 5 mm and at least 10 cm distance to each other were taken. In 6 × 10 cm depth intervals, a biopore was vertically cut in half. The roots were removed from the lumen and then the first 2 mm biopore sheath were sampled with a newly designed, self-made sampling device (Figure 1). This device has a round-shaped, sharp stainless steel blade, and is available in different sizes to allow successive sampling of soil from defined distances from the pore wall surface. Thus, using these tools, soil was sampled in 0–2, 2–4, 4–8, and 8–12 mm lateral distance from the pore. The biopores studied had diameters of 6–13 mm and presumably were formed mainly by anecic earthworm and taproots. The diameter of the biopore was measured twice, once at the top (45 cm soil depth) and once at the bottom (95 cm soil depth), to calculate the pore volume and, thus, the RLD for each distance from macropore.



(**a**) (**b**)

Figure 1. Biopore sheath scraping device: (**a**) sampling biopore sheath of 2–4 mm distance from macropore; (**b**) scraping device in different sizes.

For conservation and analytical purposes, it was necessary to dry the samples at 40 °C for four days. In a Petri dish, the roots were separated from the soil with a tweezer, while the soil was carefully crushed with a mortar pestle. The separated roots were stored overnight in a water bath and then scanned and analyzed with a scanning device (Expression 12,000 XL, Seiko Epson Corp.) and software package WinrhizoPro^{TM} 2017 by Regent Instruments, Quebec, Canada. With this procedure, root length (cm), average root diameter (mm), and root length (cm) per diameter class (mm) were measured. Four diameter classes were assessed: fine (0.0–0.2 mm), small (0.2–0.4 mm), medium (0.4–0.6 mm), and coarse (0.6–5 mm). Soil samples were sieved <2 mm, milled to fine-powder quality, and measured for total nitrogen (N_t)- and total carbon (C_t)-content with the elemental analyzer (Vario Max, Elementar, Langenselbold, Germany). To calculate the RLD (cm cm^{-3}), the measured root length (cm) was divided by the corresponding soil volume. The volume of the distances from macropore were calculated by the equation of a cylinder.

2.2. Statistical Data Analysis

Data analysis was done separately by the species faba bean and spring barley. Calculations were performed with the packages SAS/BASE and SAS/STAT 14.3 of the software SAS 9.4 TS1M5 (SAS Institute Inc., Cary, NC, USA, 2016). A hierarchical linear mixed model with fixed effects for *lateral distance*, *depth interval*, and interaction *lateral*depth*, and a random effect of *block*pore*, was fitted using restricted maximum likelihood (REML [40]), with the procedure MIXED of SAS/STAT [41]. For the variance estimate of *block*pore* and the residual error variance, heterogeneity regarding the depth intervals was allowed. In the case of convergence problems, the model was first simplified having homogeneous variance *block*pore*, and, if necessary, further simplified having homogeneous residual variance. To achieve an approximately Gaussian distribution of residuals, the RLD was transformed with the decadic logarithm, e.g.,

$$\text{logRLD} = \text{log10 (RLD + 0.01)} \quad (1)$$

The percentage of root length categorized in diameter classes *fine, small, medium, and coarse* was transformed with the logit formula, e.g.,

$$\text{logit\_Fine} = \text{log((p\_Fine + 0.005)/(1-p\_Fine + 0.005))} \quad (2)$$

Degrees of freedom for significance tests were approximated with the method of Kenward and Roger [42], and standard errors for means and differences were calculated using the method of Kackar and Harville [43], all included in the MIXED procedure.

Based on the variance estimates of the linear mixed model, multiple mean comparison tests were performed for differences between levels of lateral distances within each level of depth interval. To adjust for multiple comparisons, Tukey's honestly significant difference procedure with a family-wise error rate of $\alpha = 0.05$ was used, as described by Piepho [44].

3. Results

3.1. Root Distribution

The RLD of both crops decreased from the biopore lumen with increasing distance from macropore surface (Figure 2). For spring barley, there was a significant difference between biopore lumen + 2 mm and the other distances from macropore, except for the lowest soil depth level. Also, down to 75 cm soil depth, the RLD at 2–4 mm distance was significantly higher than at 4–8 mm and/or 8–12 mm distance. In faba bean, a significant difference in the RLD was found in 55–65 cm soil depth between the RLD in biopore lumen + 2 mm and 8–12 mm. The declining gradient of the RLD with increasing distance from the biopore lumen is more distinct for spring barley, as compared to faba bean. There was a decrease in the RLD with increasing soil depth. Roots of both crops were observed in a soil depth up to 105 cm, but less root growth of faba bean, especially in a depth of 85–105 cm. Overall, a 7.4 times higher RLD was found for spring barley (7.74 cm cm^{-3}) in lumen + 2 mm than in faba bean (1.05 cm cm^{-3}) (Supplementary Table S1).

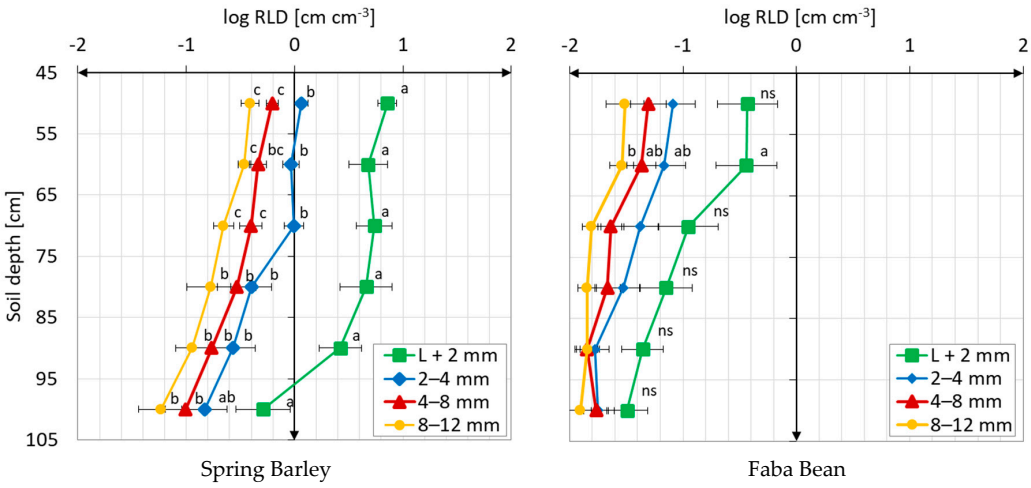


Figure 2. Log10-transformed RLD (cm cm^{-3}) of spring barley and faba bean. Mean and SE of lumen + 2 mm (L + 2) and the distances from macropore in different depth intervals. Means not sharing a common letter show significant differences between the distances from macropore within one depth interval (Tukey honestly significant difference (HSD), $\alpha = 0.05$); ns = not significant.

In both species, the highest share of roots (Table 2) was found in diameter class fine (0–0.2 mm) and small (0.2–0.4 mm). In spring barley, there was a clear difference between the share of roots in the biopore lumen + 2 mm and the further distances from macropore of fine, medium, and coarse roots. Within the diameter class, there was no significant difference between 2–4, 4–8, and 8–12 mm distance from macropore. In faba bean, there was a significant difference between the biopore lumen + 2 mm and 8–12 mm within the diameter class coarse.

Table 2. Proportion of root length (%) categorized in diameter classes (mm) of spring barley and faba bean. Mean and SE of lumen + 2 mm (L + 2) and the distances from macropore. Means not sharing a common letter indicate significant differences between the distances from macropore within one depth interval (Tukey HSD, $\alpha = 0.05$).

	Lateral Distance (mm)	Fine 0–0.2 mm (%) Mean	SE	Small 0.2–0.4 mm (%) Mean	SE	Medium 0.4–0.6 mm (%) Mean	SE	Coarse 0.6–5 mm (%) Mean	SE
Spring Barley	L + 2	43.7 ^{b}	±4.1	34.9 ^{a}	±2.9	13.4 ^{a}	±2.0	7.9 ^{a}	±1.7
	2–4	53.1 ^{a}	±5.3	38.2 ^{a}	±4.2	7.0 ^{b}	±2.1	1.8 ^{b}	±1.0
	4–8	51.3 ^{a}	±4.9	41.8 ^{a}	±4.3	5.8 ^{b}	±1.5	1.1 ^{b}	±0.6
	8–12	52.5 ^{a}	±4.6	39.5 ^{a}	±3.8	7.0 ^{b}	±1.6	1.0 ^{b}	±0.5
Faba Bean	L + 2	43.9 ^{a}	±11.9	31.3 ^{a}	±7.7	11.8 ^{a}	±4.6	13.0 ^{a}	±5.9
	2–4	62.8 ^{a}	±12.3	26.6 ^{a}	±8.3	6.6 ^{a}	±4.7	4.0 ^{ab}	±3.1
	4–8	56.0 ^{a}	±10.9	32.7 ^{a}	±9.1	6.2 ^{a}	±2.9	5.1 ^{ab}	±4.0
	8–12	53.1 ^{a}	±13.5	38.6 ^{a}	±11.7	6.1 ^{a}	±3.9	2.2 ^{b}	±1.5

The average root diameter of spring barley differs in a soil depth of 55–85 cm between the biopore lumen + 2 mm and at least one other lateral distance, with higher average diameters in biopore lumen + 2 mm (Supplementary Table S2). In faba bean, there is no significant difference between the distances from macropore within each soil depth interval, but the average root diameter is also tendentially higher in biopore lumen + 2 mm.

3.2. N_t-, C_t-Content and CN-Ratio

There is a lateral decrease in N_t-, C_t-content and CN-ratio from the biopore lumen towards 8–12 mm, as well as a decrease with increasing soil depth (Table 3). The N_t- and C_t-contents in 0–2 mm are significantly higher compared to 8–12 mm lateral distance from macropore across the soil depth. In contrast, the N_t- and C_t-contents in 4–8 mm distance from the pore were not different from 8–12 mm, with one exception in the N_t-content, at 85–95 cm soil depth in the barley plots. The CN-ratio was significantly higher in 0–2 mm than at 4–8 or 8–12 mm distance from macropore at most depth levels.

Table 3. Lateral variations of N_t-, C_t-content and CN-ratio from biopore lumen to 8–12 mm. Mean and SE of the distances from macropore in different depth intervals. Means not sharing a common letter indicate significant differences between the distances from macropore within one depth interval (Tukey HSD, $\alpha = 0.05$).

		Spring Barley						Faba Bean					
Soil Depth	Lateral Distance	N_t (%)		C_t (%)		C/N		N_t (%)		C_t (%)		C/N	
(cm)	(mm)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
45–55	0–2	0.070 ^{a}	±0.003	0.65 ^{a}	±0.02	9.3 ^{a}	±0.2	0.062 ^{a}	±0.003	0.55 ^{a}	±0.03	8.8 ^{a}	±0.2
	2–4	0.060 ^{b}	±0.003	0.52 ^{b}	±0.02	8.8 ^{ab}	±0.2	0.051 ^{b}	±0.001	0.43 ^{ab}	±0.01	8.4 ^{ab}	±0.2
	4–8	0.056 ^{c}	±0.002	0.47 ^{b}	±0.01	8.5 ^{b}	±0.3	0.047 ^{b}	±0.001	0.39 ^{b}	±0.01	8.2 ^{b}	±0.2
	8–12	0.055 ^{c}	±0.002	0.46 ^{b}	±0.01	8.5 ^{b}	±0.3	0.046 ^{b}	±0.001	0.38 ^{b}	±0.01	8.1 ^{b}	±0.1
55–65	0–2	0.068 ^{a}	±0.003	0.61 ^{a}	±0.02	8.9 ^{a}	±0.2	0.060 ^{a}	±0.002	0.52 ^{a}	±0.03	8.6 ^{a}	±0.2
	2–4	0.058 ^{b}	±0.002	0.50 ^{b}	±0.02	8.5 ^{ab}	±0.3	0.052 ^{b}	±0.002	0.44 ^{ab}	±0.03	8.5 ^{ab}	±0.4
	4–8	0.056 ^{c}	±0.001	0.46 ^{b}	±0.01	8.2 ^{b}	±0.3	0.049 ^{b}	±0.001	0.39 ^{b}	±0.01	8.0 ^{b}	±0.1
	8–12	0.054 ^{c}	±0.001	0.44 ^{b}	±0.01	8.2 ^{b}	±0.3	0.048 ^{b}	±0.001	0.38 ^{b}	±0.01	7.9 ^{b}	±0.1
65–75	0–2	0.068 ^{a}	±0.002	0.61 ^{a}	±0.02	9.1 ^{a}	±0.2	0.059 ^{a}	±0.002	0.50 ^{a}	±0.03	8.4 ^{a}	±0.2
	2–4	0.056 ^{b}	±0.001	0.45 ^{b}	±0.01	8.2 ^{b}	±0.2	0.053 ^{ab}	±0.002	0.43 ^{ab}	±0.02	8.1 ^{a}	±0.2
	4–8	0.052 ^{bc}	±0.001	0.40 ^{c}	±0.01	7.7 ^{bc}	±0.2	0.050 ^{b}	±0.001	0.38 ^{b}	±0.01	7.8 ^{a}	±0.2
	8–12	0.050 ^{c}	±0.001	0.38 ^{c}	±0.01	7.6 ^{c}	±0.2	0.048 ^{b}	±0.002	0.37 ^{b}	±0.01	7.5 ^{a}	±0.2
75–85	0–2	0.068 ^{a}	±0.002	0.59 ^{a}	±0.03	8.6 ^{a}	±0.2	0.058 ^{a}	±0.002	0.50 ^{a}	±0.03	8.5 ^{a}	±0.2
	2–4	0.054 ^{b}	±0.001	0.41 ^{b}	±0.01	7.7 ^{b}	±0.1	0.051 ^{ab}	±0.002	0.41 ^{ab}	±0.02	8.0 ^{ab}	±0.3
	4–8	0.051 ^{c}	±0.001	0.38 ^{b}	±0.01	7.4 ^{b}	±0.2	0.048 ^{bc}	±0.001	0.37 ^{b}	±0.01	7.7 ^{b}	±0.3
	8–12	0.050 ^{c}	±0.001	0.37 ^{b}	±0.01	7.3 ^{b}	±0.2	0.046 ^{c}	±0.001	0.35 ^{b}	±0.01	7.7 ^{b}	±0.3

Table 3. *Cont.*

Soil Depth	Lateral Distance	Spring Barley						Faba Bean					
		N_t (%)		C_t (%)		C/N		N_t (%)		C_t (%)		C/N	
(cm)	(mm)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
85–95	0–2	0.064 ^{a}	±0.002	0.57 ^{a}	±0.03	9.0 ^{a}	±0.5	0.056 ^{a}	±0.002	0.48 ^{a}	±0.03	8.4 ^{a}	±0.3
	2–4	0.051 ^{b}	±0.001	0.43 ^{ab}	±0.03	8.3 ^{b}	±0.6	0.048 ^{ab}	±0.001	0.38 ^{ab}	±0.02	7.9 ^{b}	±0.3
	4–8	0.048 ^{b}	±0.001	0.37 ^{b}	±0.02	7.6 ^{b}	±0.4	0.045 ^{bc}	±0.001	0.35 ^{b}	±0.01	7.7 ^{b}	±0.2
	8–12	0.045 ^{c}	±0.001	0.35 ^{b}	±0.02	7.7 ^{b}	±0.4	0.044 ^{c}	±0.001	0.33 ^{b}	±0.01	7.6 ^{b}	±0.3
95–105	0–2	0.060 ^{a}	±0.002	0.53 ^{a}	±0.04	8.7 ^{a}	±0.3	0.056 ^{a}	±0.002	0.48 ^{a}	±0.03	8.6 ^{a}	±0.3
	2–4	0.047 ^{b}	±0.002	0.39 ^{ab}	±0.02	8.1 ^{b}	±0.4	0.047 ^{b}	±0.001	0.37 ^{ab}	±0.01	7.8 ^{b}	±0.3
	4–8	0.042 ^{c}	±0.002	0.33 ^{b}	±0.01	7.2 ^{c}	±0.1	0.045 ^{b}	±0.001	0.34 ^{b}	±0.01	7.6 ^{bc}	±0.3
	8–12	0.040 ^{c}	±0.002	0.32 ^{b}	±0.01	7.3 ^{c}	±0.2	0.044 ^{b}	±0.001	0.32 ^{b}	±0.01	7.5 ^{c}	±0.3

4. Discussion

The observed lateral gradient of the RLD at time of sampling implies that roots of spring barley and faba bean elongate through biopores and are able to enter the biopore sheath. They can then possibly explore further subsoil areas. That aligns with Athmann et al. [45], who observed, in a pot experiment by in situ endoscopy images, that around 20–25% of the biopore-grown roots of spring barley and faba bean penetrated the biopore wall. Hence, it seems plausible that the biopore lumen not only serves as a propagation path for roots, but that the pore wall is also permeable enough to allow root entry into the biopore sheath. However, the results show a preferential root growth in the biopore lumen + 2 mm, less in 2–4 mm, and rarely in 4–8 and 8–12 mm distance from macropore. This decrease indicates either young roots, which just entered the sheath, or a higher proportion of roots remaining in the initial millimeters. That can be due to a nutrient enrichment at the inner layer of the sheath and/or the sheath as a limiting root growing environment. Young roots at flowering support nutrient acquisition. This may be important, particularly in low-input farming systems where crops are more dependent on nutrient acquisition from the solid soil phase at deeper soil layers.

The inner layer of the biopore sheath seems to be a favorable growing environment for roots in subsoil, as indicated by physical accessibility and a higher N_t-content, as compared to the lateral distance of 8–12 mm. On the other hand, the sheath can impede root growth due to an impeding soil particle arrangement with less pore connectivity [35,36]. Impeded root growth is indicated by less root elongation, increased tortuosity [46], and radial root growth identifiable by a larger root diameter [37,38,47]. The overall mean RLD of 7.74 cm cm^{-3} in the pore lumen + 2 mm over the sampled soil depth for spring barley is relatively high in comparison to previous studies, with 1.5 cm cm^{-3} RLD of winter barley within the pore (same soil depth and experimental side, but using a different method and possible different subsoil moisture) [48,49]. This indicates that, in this study, the subsoil roots of spring barley colonized the biopores due to a hard setting soil environment. As Atkinson et al. [50] have reported, colonization of macropores is an important strategy of wheat roots to compacted subsoil. Contrary to homorhizous barley, allorhizous faba bean, with its thicker roots [51], less plasticity [52], and independance from nitrogen in the sheath, might be less attracted by the biopore sheath. Despite that both species dispose of a different root system architecture, the 7.4-times higher RLD in lumen + 2 mm directs the assumption that barley used the inner layer of the biopore sheath more efficiently than faba bean. That can be due to the N-enrichment and a higher dependence of pathways with less mechanical impedance while growing through subsoil.

With roots entering the biopore sheath, particularly the proportion of fine and small roots will be important for nutrient uptake in an enriched compartment within subsoil. The study revealed differences in root diameters between the biopore lumen + 2 mm and the other sampling zones (Table 2). Commonly it is assumed that root diameter decreases with increasing root differentiation, but due to technical reasons, root branching could not be measured as part of this study. However, endoscopy images by Athmann et al. [20] showed that 54% (barley) or 66% (oilseed rape) of all roots in biopores established contact to the pore wall via lateral roots, which supports the view that a large share of roots in the biopore sheath are roots of higher orders. Moreover, Zhan and Lynch [53] reported that

sparse but long lateral roots promote N capture in soils with low N-content. Root branching depends on genotype specific plasticity [54] and is influenced by soil compaction [55]. According to this, it can be assumed that in the current study, especially for barley, the sharply decreased RLD in the sheath is due to mainly lateral roots entering the sheath.

Apart from that, two contrasting physical stresses can influence root diameter generally: when roots are exposed to radial pressure, root diameter can be reduced; by contrast, when roots are exposed to axial pressure, their diameter can largely increase [37]. Since the highest root diameter was found in the biopore lumen + 2 mm, it therefore seems plausible that the absence of radial pressure on roots growing through the pore lumen allowed for unimpeded radial root enlargement. In addition, it is possible that the biopore wall exerted axial stresses on roots while penetrating the biopore sheath from the lumen, which resulted in root thickening. This view is in line with the results of Han et al. [49], who investigated winter barley roots at the same study site. They discovered a higher proportion of coarse roots and a lower proportion of fine roots when barley was grown in field plots with increased density of large-sized biopores. Furthermore, the high share of fine and small roots until 12 mm distance from macropore in both species can be supported by the presence of small lateral pores in the sheath of macropores. Such pores have been found by Pagenkemper et al. [36] with computed tomography, but to date there is no study addressing to what extent occurrence and status of these lateral pores is decisive for root growth in the biopore sheath. Accordingly, it can be suggested that in the current study, entering the biopore sheath was facilitated by lateral pores, because of the less RLD within the biopore sheath in comparison to lumen + 2 mm.

The lateral decrease of the N_t- and C_t-content in the biopore sheath may be caused by earthworm activity and root growth. Anecic earthworms remove litter from the soil surface into their burrow, digest it, and then coat the biopore wall. Plant roots are growing along biopores as well as entering the biopore sheath. They leave rhizodeposits while growing, and organic matter while decaying. While growing, roots establish contact with both dead and living roots in biopores, and these are strongly colonized by microorganisms [56]. Microbial interactions are formed more quickly in biopores than in the bulk soil [57]. Thus, there is significant potential for N_t-turnover [58]. Therefore, a potentially higher N-uptake from biopore wall and 2–4 mm sheath can help to explain the decreasing CN-ratio from pore surface to 8–12 mm distance from macropore. However, it is still questionable whether crops can acquire any relevant amount of nutrients by growing along the biopore wall or by entering the biopore sheath, respectively.

5. Conclusions

In the current study, a quantitative investigation of the RLD in thin layers of the biopore sheath in arable subsoil was carried out. Roots of both crops under study were grown through the biopore sheath. The relevance of the biopore sheath for root growth appears to be higher for barley than for faba bean. The high proportion of fine and small roots, along with the smaller average root diameter of roots growing in the biopore sheath, indicate no major axial impedance, and that the roots possibly followed lateral pores. The higher RLD and N_t-content in the closer surroundings to the biopore, than at 8–12 mm distance, support the view that the inner layer of the sheath is a "hotspot" of nutrient acquisition in the subsoil, especially for barley. In light of the comparatively small total volume of the layers of the biopore sheath, it remains uncertain to what extent they contribute to nutrient uptake. Since organic agriculture aims to make naturally available resources accessible, it should be further investigated whether the surroundings of biopores can be relevant sources of nutrients.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0472/10/12/650/s1, Table S1. Root length density (RLD) in (cm cm^{-3}) of spring barley and faba bean. Mean and SE of the distances from macropore at each depth interval. Table S2. Average root diameter (mm) of spring barley and faba bean. Mean and SE of the distances from macropore at each depth interval. Means not sharing a common letter indicate significant differences between the distances from macropore within a depth interval (Tukey HSD, $\alpha = 0.05$). In faba bean there is no significant difference.

Author Contributions: Project administration by T.K.; T.K. and M.A. conceptualized the study, including the idea and design of the experiment. L.P. performed the experiment in the field. L.P. and A.B. analyzed the data. L.P. wrote the article, and T.K., M.A., and A.B. supported the data interpretation, reviewed, and edited the writing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare that they have no conflicts of interest.

References

1. Hodge, A.; Berta, G.; Doussan, C.; Merchan, F.; Crespi, M. Plant root growth, architecture and function. *Plant Soil* **2009**, *321*, 153–187. [CrossRef]
2. Kutschera, L.; Lichtenegger, E.; Sobotik, M. *Hordeum vulgare* L., vielzeilige gerste. In *Wurzelatlas der Kulturpflanzen Gemäßigter Gebiete Mit Arten des Feldgemüseanbaus*; DLG-Verlags-GmbH: Frankfurt am Main, Germany, 2009; Volume 7, p. 212.
3. Amelung, W.; Blume, H.-P.; Fleige, H.; Horn, R.; Kandeler, E.; Kögel-Knabner, I.; Kretzschmar, R.; Stahr, K.; Wilke, B.-M. Böden als pflanzenstandorte. In *Scheffer/Schachtschabel Lehrbuch der Bodenkunde*, 17th ed.; Springer-Verlag GmbH: Berlin/Heidelberg, Germany, 2018; pp. 491–581. [CrossRef]
4. Gaiser, T.; Perkons, U.; Küpper, P.M.; Uteau Puschmann, D.; Peth, S.; Kautz, T.; Pfeifer, J.; Ewert, F.; Horn, R.; Köpke, U. Evidence of improved water uptake from subsoil by spring wheat following lucerne in a temperate humid climate. *Field Crops Res.* **2012**, *126*, 56–62. [CrossRef]
5. Fleige, H.; Grimme, H.; Renger, M.; Strebel, O. Zur erfassung der nährstoffanlieferung durch diffusion im effektiven wurzelraum. *Mitt. Dtsch. Bodenkd. Ges.* **1983**, *38*, 381–386.
6. Kuhlmann, H.; Baumgärtel, G. Potential importance of the subsoil for the P and Mg nutrition of wheat. *Plant Soil* **1991**, *137*, 259–266. [CrossRef]
7. Ehlers, W.; Köpke, U.; Hesse, F.; Böhm, W. Penetration resistance and root growth of oats in tilled and untilled loess soil. *Soil Tillage Res.* **1983**, *3*, 261–275. [CrossRef]
8. Kautz, T.; Amelung, W.; Ewert, F.; Gaiser, T.; Horn, R.; Jahn, R.; Javaux, M.; Kemna, A.; Kuzyakov, Y.; Munch, J.C.; et al. Nutrient acquisition from arable subsoils in temperate climates: A review. *Soil Biol. Biochem.* **2013**, *57*, 1003–1022. [CrossRef]
9. Kuzyakov, Y.; Blagodatskaya, E. Microbial hotspots and hot moments in soil: Concept & review. *Soil Biol. Biochem.* **2015**, *83*, 184–199. [CrossRef]
10. Tiunov, A.V.; Scheu, S. Microbial respiration, biomass, biovolume and nutrient status in burrow walls of *Lumbricus terrestris* L. (Lumbricidae). *Soil Biol. Biochem.* **1999**, *31*, 2039–2048. [CrossRef]
11. Uksa, M.; Schloter, M.; Kautz, T.; Athmann, M.; Köpke, U.; Fischer, D. Spatial variability of hydrolytic and oxidative potential enzyme activities in different subsoil compartments. *Biol. Fertil. Soils* **2015**, *51*, 517–521. [CrossRef]
12. Hagedorn, F.; Bundt, M. The age of preferential flow paths. *Geoderma* **2002**, *108*, 119–132. [CrossRef]
13. Perkons, U.; Kautz, T.; Uteau, D.; Peth, S.; Geier, V.; Thomas, K.; Lütke Holz, K.; Athmann, M.; Pude, R.; Köpke, U. Root-length densities of various annual crops following crops with contrasting root systems. *Soil Tillage Res.* **2014**, *137*, 50–57. [CrossRef]
14. Kautz, T.; Perkons, U.; Athmann, M.; Pude, R.; Köpke, U. Barley roots are not constrained to large-sized biopores in the subsoil of a deep Haplic Luvisol. *Biol. Fertil. Soils* **2013**, *49*, 959–963. [CrossRef]
15. Stewart, J.B.; Moran, C.J.; Wood, J.T. Macropore sheath: Quantification of plant root and soil macropore association. *Plant Soil* **1999**, *211*, 59–67. [CrossRef]
16. Pierret, A.; Moran, C.J.; Pankhurst, C.E. Differentiation of soil properties related to the spatial association of wheat roots and soil macropores. *Plant Soil* **1999**, *211*, 51–58. [CrossRef]
17. White, R.G.; Kirkegaard, J.A. The distribution and abundance of wheat roots in a dense, structured subsoil – implications for water uptake. *Plant Cell Environ.* **2010**, *33*, 133–148. [CrossRef]

18. Stirzaker, R.J.; Passioura, J.B.; Wilms, Y. Soil structure and plant growth: Impact of bulk density and biopores. *Plant Soil* **1996**, *185*, 151–162. [CrossRef]
19. Passioura, J.B. Soil structure and plant growth. *Aust. J. Soil Res.* **1991**, *29*, 717–728. [CrossRef]
20. Athmann, M.; Kautz, T.; Pude, R.; Köpke, U. Root growth in biopores—Evaluation with in situ endoscopy. *Plant Soil* **2013**, *371*, 179–190. [CrossRef]
21. Bouché, M.B. Action de la faune sur les états de la matière organique dans les ecosystèmes. In *Humification et Biodégradation*; Kilbertius, G., Reisinger, O., Mourey, A., Cancela da Fonseca, J.A., Eds.; Pierron: Sarreguemines, France, 1975; pp. 157–168.
22. Brown, G.G.; Baroisa, I.; Lavelle, P. Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. *Eur. J. Soil Biol.* **2000**, *36*, 177–198. [CrossRef]
23. Lavelle, P. Earthworm activities and the soil system. *Biol. Fertil. Soils* **1988**, *6*, 237–251. [CrossRef]
24. Pankhurst, C.E.; Pierret, A.; Hawke, B.G.; Kirby, J.M. Microbiological and chemical properties of soil associated with macropores at different depths in a red-duplex soil in NSW Australia. *Plant Soil* **2002**, *238*, 11–20. [CrossRef]
25. Athmann, M.; Kautz, T.; Banfield, C.; Bauke, S.; Hoang, D.T.T.; Lüsebrink, M.; Pausch, J.; Amelung, W.; Kuzyakov, Y.; Köpke, U. Six months of *L. terrestris* L. activity in root-formed biopores increases nutrient availability, microbial biomass and enzyme activity. *Appl. Soil Ecol.* **2017**, *120*, 135–142. [CrossRef]
26. Don, A.; Steinberg, B.; Schöning, I.; Pritsch, K.; Joschko, M.; Gleixner, G.; Schulze, E.-D. Organic carbon sequestration in earthworm burrows. *Soil Biol. Biochem.* **2008**, *40*, 1803–1812. [CrossRef]
27. Tiunov, A.V.; Bonkowski, M.; Alphei, J.; Scheu, S. Microflora, Protozoa and Nematoda in Lumbricus terrestris burrow walls: A laboratory experiment. *Pedobiologia* **2001**, *45*, 46–60. [CrossRef]
28. Jégou, D.; Cluzeaua, D.; Hallaireb, V.; Balesdentc, J.; Tréhen, P. Burrowing activity of the earthworms *Lumbricus terrestris* and *Aporrectodea giardi* and consequences on C transfers in soil. *Eur. J. Soil Biol.* **2000**, *36*, 27–34. [CrossRef]
29. Andriuzzi, W.; Bolger, T.; Schmidt, O. The drilosphere concept: Fine-scale incorporation of surface residue-derived N and C around natural Lumbricus terrestris burrows. *Soil Biol. Biochem.* **2013**, *64*, 136–138. [CrossRef]
30. Bengough, A.G. Root Growth and function in relation to soil structure, composition, and strength. In *Root Ecology*; de Kroon, H., Visser, E.J.W., Eds.; Springer: Berlin/Heidelberg, Germany, 2003; pp. 151–171.
31. Uteau, D.; Pagenkemper, S.K.; Peth, S.; Horn, R. Root and time dependent soil structure formation and its influence on gas transport in the subsoil. *Soil Tillage Res.* **2013**, *132*, 69–76. [CrossRef]
32. Lee, K.E. *Earthworms: Their Ecology and Relationship with Soil and Land Use*; Academic Press: Sydney, Australia, 1985.
33. Hinsinger, P.; Bengough, A.G.; Vetterlein, D.; Young, I.M. Rhizosphere: Biophysics, biogeochemistry and ecological relevance. *Plant Soil* **2009**, *321*, 117–152. [CrossRef]
34. Helliwell, J.R.; Sturrock, C.J.; Mairhofer, S.; Craigon, J.; Ashton, R.W.; Miller, A.J.; Whalley, W.R.; Mooney, S.J. The emergent rhizosphere: Imaging the development of the porous architecture at the root-soil interface. *Sci. Rep.* **2017**, *7*, 1–10. [CrossRef]
35. Schrader, S.; Rogasik, H.; Onasch, I.; Jégou, D. Assessment of soil structural differentiation around earthworm burrows by means of X-ray computed tomography and scanning electron microscopy. *Geoderma* **2007**, *137*, 378–387. [CrossRef]
36. Pagenkemper, S.K.; Athmann, M.; Uteau, D.; Kautz, T.; Peth, S.; Horn, R. The effect of earthworm activity on soil bioporosity—Investigated with X-ray computed tomography and endoscopy. *Soil Tillage Res.* **2015**, *146*, 79–88. [CrossRef]
37. Bengough, A.G. Root elongation is restricted by axial but not by radial pressures: So what happens in field soil? *Plant Soil* **2012**, *360*, 15–18. [CrossRef]
38. Kolb, E.; Hartmann, C.; Genet, P. Radial force development during root growth measured by photoelasticity. *Plant Soil* **2012**, *360*, 19–35. [CrossRef]
39. Pätzold, S.; Vetterlein, D.; Jahn, R. DFG Research Unit 1320. *Crop Sequence and the Nutrient Acquisition from the Subsoil. Description of the Reference Soil Profile*. Available online: https://www.cka.uni-bonn.de/standort/bodenprofilbeschreibung-cka (accessed on 12 November 2020).

40. Patterson, H.D.; Thompson, R. Recovery of inter-block information when block sizes are unequal. *Biometrika* **1971**, *58*, 545–554. [CrossRef]
41. Littell, R.C.; Milliken, G.A.; Stroup, W.W.; Wolfinger, R.D.; Schabenberger, O. *SAS for Mixed Model*, 2nd ed.; SAS Institute Inc.: Cary, NC, USA, 2006.
42. Kenward, M.G.; Roger, J.R. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* **1997**, *53*, 983–997. [CrossRef]
43. Kackar, R.N.; Harville, D.A. Approximations for standard errors of estimators of fixed and random effects in mixed linear models. *J. Am. Stat. Assoc.* **1984**, *388*, 853–862.
44. Piepho, H. A SAS macro for generating letter displays of pairwise mean comparisons. *Commun. Biometry Crop Sci.* **2012**, *1*, 4–13.
45. Athmann, M.; Sondermann, J.; Kautz, T.; Köpke, U. Comparing macropore exploration by Faba bean, wheat, barley and oilseed rape roots using in situ endoscopy. *J. Soil Sci. Plant Nutr.* **2019**, *19*, 689–700. [CrossRef]
46. Popova, L.; Van Dusschoten, D.; Nagel, K.A.; Fiorani, F.; Mazzolai, B. Plant root tortuosity: An indicator of root path formation in soil with different composition and density. *Ann. Bot.* **2016**, *118*, 685–698. [CrossRef]
47. Lynch, J.; Marschner, P.; Rengel, Z. Effect of internal and external factors on root growth and development. In *Marschner's Mineral Nutrition of Higher Plants*, 3rd ed.; Marschner, P., Ed.; Academic Press: San Diego, CA, USA, 2012; pp. 331–346.
48. Perkons, U. Bioporengenese Durch Homo- und Allorhize Kulturpflanzen: Einfluss auf das Wurzelwachstum der Nachfrüchte. Ph.D. Thesis, University of Bonn, Bonn, Germany, 2018.
49. Han, E.; Kautz, T.; Köpke, U. Precrop root system determines root diameter of subsequent crop. *Biol. Fertil. Soils* **2016**, *52*, 113–118. [CrossRef]
50. Atkinson, J.A.; Hawkesford, M.J.; Whalley, W.R.; Zhou, H.; Mooney, S.J. Soil strength influences wheat root interactions with soil macropores. *Plant Cell Environ.* **2020**, *43*, 235–245. [CrossRef] [PubMed]
51. Materechera, S.A.; Dexter, A.R.; Alston, A.M. Penetration of very strong soils by seedling roots of different species. *Plant Soil* **1991**, *135*, 31–41. [CrossRef]
52. Li, H.B.; Ma, Q.H.; Li, H.G.; Zhan, F.S.; Rengel, Z.; Shen, J.B. Root morphological responses to localized nutrient supply differ among crop species with contrasting root traits. *Plant Soil* **2014**, *376*, 151–163. [CrossRef]
53. Zhan, A.; Lynch, J.P. Reduced frequency of lateral root branching improves N capture from low-N soils in maize. *J. Exp. Bot.* **2015**, *66*, 2055–2065. [CrossRef] [PubMed]
54. Colombi, T.; Walter, A. Genetic diversity under soil compaction in wheat: Root number as a promising trait for early plant vigor. *Front. Plant Sci.* **2017**, *8*, 1–14. [CrossRef] [PubMed]
55. Correa, J.; Postma, J.A.; Watt, M.; Wojciechowski, T. Soil compaction and the architectural plasticity of root systems. *J. Exp. Bot.* **2019**, *70*, 6019–6034. [CrossRef]
56. Watt, M.; Hugenholtz, P.; White, R.; Vinall, K. Numbers and locations of native bacteria on field-grown wheat roots quantified by fluorescence in situ hybridization (FISH). *Environ. Microbiol.* **2006**, *8*, 871–884. [CrossRef]
57. Watt, M.; Silk, W.K.; Passioura, J.B. Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. *Ann. Bot.* **2006**, *97*, 839–855. [CrossRef]
58. Jones, D.L.; Magthab, E.A.; Gleeson, D.B.; Hill, P.W.; Sánchez-Rodríguez, A.R.; Roberts, P.; Ge, T.; Murphy, D.V. Microbial competition for nitrogen and carbon is as intense in the subsoil as in the topsoil. *Soil Biol. Biochem.* **2018**, *117*, 72–82. [CrossRef]