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Biopore-Induced Deep Root Traits of Two Winter Crops

Ning Huang [1,2], **Miriam Athmann** [1] **and Eusun Han** [2,*]

[1] Department of Agroecology and Organic Farming, Faculty of Agriculture, University of Bonn, Auf dem Hügel 6, 53121 Bonn, Germany; nih@plen.ku.dk (N.H.); mathmann@uni-bonn.de (M.A.)
[2] Department of Plant and Environmental Sciences, University of Copenhagen, Højbakkegård Alle 13, 2630 Taastrup, Denmark
* Correspondence: eusun.han@plen.ku.dk

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Abstract: Deeper root growth can be induced by increased biopore density. In this study, we aimed to compare deep root traits of two winter crops in field conditions in response to altered biopore density as affected by crop sequence. Two fodder crop species—chicory and tall fescue—were grown for two consecutive years as preceding crops (pre-crops). Root traits of two winter crops—barley and canola, which were grown as subsequent crops (post-crops)—were measured using the profile wall and soil monolith method. While barley and canola differed greatly in deep root traits, they both significantly increased rooting density inside biopores by two-fold at soil depths shallower than 100 cm. A similar increase in rooting density in the bulk soil was observed below 100 cm soil depth. As a result, rooting depth significantly increased (>5 cm) under biopore-rich conditions throughout the season of the winter crops. Morphological root traits revealed species-wise variation in response to altered biopore density, in which only barley increased root size under biopore-rich conditions. We concluded that large-sized biopores induce deeper rooting of winter crops that can increase soil resource acquisition potential, which is considered to be important for agricultural systems with less outsourced farm resources, e.g., Organic Agriculture. Crops with contrasting root systems can respond differently to varying biopore density, especially root morphology, which should be taken into account upon exploiting biopore-rich conditions in arable fields. Our results also indicate the need for further detailed research with a greater number of species, varieties and genotypes for functional classification of root plasticity against the altered subsoil structure.

Keywords: biopores; root traits; pre-crops; winter crops; deep roots; PCA; root plasticity

1. Introduction

In the face of climate change and the ever-increasing demand for sustainable intensification, more resilient and resource use-efficient crop production is called for [1]. A neglected strategy is inducing crops' root traits for deeper and better resource exploitation [2]. Winter crops, with their longer crop phenology, can grow deeper roots than short-season crops [3]. The deeper and longer root occupancy of winter crops can reduce nitrate leaching during the winter time [4], as well as induce better uptake of nutrients and water in following season [5,6].

One well-established strategy to induce further deeper rooting of the long season crops is early sowing, which increases rooting depth and invokes timely uptake of available resources at depth [4,7]. Crop sequence management can also trigger a rapid establishment of deeper roots of subsequent crops by cultivation of pre-crops with high bioporing capacity. It has been reported that the taprooted cover crops such as lucerne and chicory with large root sizes are more capable of forming biopores larger than 2 mm in size compared with fibrous-rooted tall fescue [8]. Subsequently grown spring crops [9], as well as winter crops [10], exhibited more rapid and deeper establishment of root systems in response to increased biopore density.

Crop species with contrasting root systems construct roots in different ways. Monocots with fibrous root systems such as cereals tend to develop greater root lengths compared with dicots due to the need to generate new xylem and phloem tissues for transportation of water and sugar, respectively [11]. This is mainly due to the absence of vascular cambium in the roots of monocots [11]. In contrast to monocots, dicots such as brassicas, with their distinctive single primary axile roots (taproot), and vascular cambia have sufficient vascular capacities that make the root systems less dense than monocots. As a result, the strategies of monocots and dicots for resource acquisition can vary, with the former requiring more roots than the latter for uptake of resources per unit length [12]. However, it is not well known how the altered subsoil structure with elevation of biopore density can affect the developmental differences of monocots and dicots. A recent qualitative study by Athmann et al. [13] with an endoscopic observation on root growth in biopore channels revealed that barley and canola explored the soil channels differently. While the roots of the former were in direct contact with the pore walls without much lateral proliferation, the latter produced more lateral roots in order to be in contact with the pore walls. Perkons et al. [10] measured root length density of different crop species (wheat, barley and canola) that exhibited effects according to individual species. However, those studies did not attempt to quantitatively measure how different crops respond to altered biopore density.

One of the most frequently investigated root traits in biopore-associated root studies has been the root length inside biopores and in the bulk soil, which has revealed varying effects over the season and along the soil depth. For example, spring wheat's root length was higher when grown under biopore-rich treatments both inside and outside biopores at early growth stages [9]. The total root length density (RLD) of winter barley also exhibited the effects of elevated biopore density only below 105 cm of soil depth [10].

Root morphology determines plants' root penetration [14] and adsorptive surface area upon nutrient acquisition [15], which greatly varies between crop species and soil conditions. However, only a few studies have investigated root morphological traits against altered biopore density. One example is the study by Han et al. [16], which demonstrated increased root diameter of barley under higher biopore density treatments. The authors claimed that the potentially reduced radial pressure inside large pores might have induced the thickening of roots upon elongation and expansion [17]. However, no detailed comparisons in root morphology between the arable crop species as affected by altered biopore density have been made so far.

Magnitude and direction of genotypic variation in root plasticity often depends on root traits. For example, density-based traits (e.g., root length) were found to show genotypic variation in plasticity but morphological traits such as specific root length (SRL) did not reveal such variation [18,19]. Recent approaches using multivariate analysis (e.g., principal component analysis: PCA) to classify phenotypic expression of different genotypes of cover crops and cereals emphasized that meaningful root classification requires multiple root traits comprising both rooting density and morphology [20]. Such an approach can be a challenge for biopore-induced root traits as they vary considerably depending on the time of observation during the season [9] as well as the soil depth [10], which requires a well-planned combination of the acquired dataset. If successful, more comprehensive understanding of roots' responses against altered subsoil structure can be acquired, which can facilitate crop sequence management and the formulation of breeding programs for better and deeper exploitation of resources.

Therefore, we aimed to (1) quantitatively compare the root traits of two winter crop species—barley and canola—in arable subsoil under varying soil biopore density as affected by crop sequence and to (2) classify the crop sequence managements based on the biopore-induced multiple root traits. We hypothesized that (i) both winter crop species would respond to the altered biopore density by increasing rooting depth and rooting density. However, (ii) the response in terms of root morphology would differ between the two crop species owing to their contrasting root system architecture and root growth patterns inside biopores. Also, (iii) the influence of varying biopore density on crops' root growth affected by crop sequence could be classified using multivariate analysis on multiple root traits.

To test the hypotheses, we created contrasting biopore density levels through cultivation of taprooted (chicory) and fibrous-rooted (tall fescue) pre-crops, after which subsequent winter crops (barley and canola) with contrasting root system architectures were grown and their root traits were measured.

2. Materials and Methods

2.1. Experimental Site

The field experiment was conducted from 2010–2013 at the Campus Klein-Altendorf (CKA) research station located near Meckenheim, Germany (50°37′9′′ N, 6°59′29′′ E). The soil was classified as Haplic Luvisol developed from loess. According to the record, from 1956 to 2005 the mean annual precipitation was 599 mm and mean temperature was 9.3 °C in the region. Monthly records of precipitation and atmospheric and soil temperature at the study site from 2012 to 2013 are shown in Figure 1.

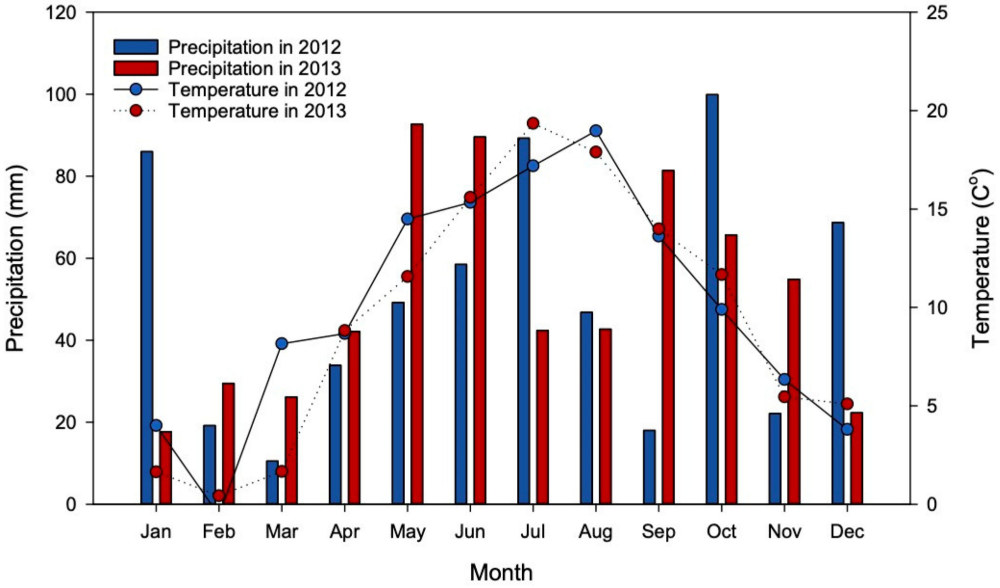

Figure 1. Monthly precipitation and atmospheric and soil temperature at the study site from 2012–2013.

2.2. Treatments

The experiment was divided into two phases called pre-cropping (2010–2011) and post-cropping phases (2012–2013; Table 1). At the first phase of the experiment, two fodder crop species, chicory (*Cichorium intybus* L. ″Puna″) with a taproot system and tall fescue (*Festuca arundinacea* Schreb. "Hykor") with a fibrous root system, were grown for two consecutive years as preceding crop species (pre-crop). Investigation was done on subsequently grown winter barley (*Hordeum vulgare* L. ″Highlight″) and winter canola (*Brassica napus* L. ″Visby″) in 2013 (post-crop). Spring wheat (*Triticum aestivum* L. ″Scirocco″) was grown in between the two phases.

Table 1. Crop sequence management at the study site from 2010 to 2013.

Crop Sequence	**Pre-Cropping Phase**		**Post-Cropping Phase**	
	2010	**2011**	**2012**	**2013**
Chicory-Barley	Chicory	Chicory	Spring wheat	Winter barley
Chicory-Canola	Chicory	Chicory	Spring wheat	Winter canola
Tall fescue-Barley	Tall fescue	Tall fescue	Spring wheat	Winter barley
Tall fescue-Canola	Tall fescue	Tall fescue	Spring wheat	Winter canola

2.3. *Agronomy*

Chicory and tall fescue were sown in April 2010 with sowing densities of 5 kg ha^{-1} and 30 kg ha^{-1}, respectively. Three cuts in 2010 and five cuts in 2011 were done on both pre-crops during the vegetative period and the shoot materials were mulched at site. No fertilizer application was done during the pre-cropping phase except for 50 kg N ha^{-1} that were added as calcium ammonium nitrate after sowing. Spring wheat was sown in March 2012 with a sowing density of 400 grains m^{-2} and a row width of 12 cm, which was harvested in early August 2012. Subsequently, winter barley and winter canola were sown in late August 2012 and early October 2012 with sowing densities of 330 and 100 kernel m^{-2}, respectively. Both crops were sown with 12 cm width intervals. All the post-crop species received 40 kg N ha^{-1} per growing season. The post-crops were harvested in July 2013. The plot size was 6 m × 10 m.

2.4. *Biopore Investigation*

Prior to sowing of spring wheat in 2012, rectangular trenches (50 cm × 50 cm) were formed to a soil depth of 45 cm. At this depth, surface area was flattened horizontally, and vacuum cleaned. The exposed surface area was photographed with a Panasonic DMC-TZ10 (2141 × 2141 pixels: 180 DPI). The biopore images were analyzed for biopore size distribution (2 mm to 10 mm at each 1 mm interval) using the Bersoft IMAGE Measurement Software (64-bit). To calibrate the biopore size in pixel numbers, four objects with a known size (10 mm × 10 mm) were placed at each corner of the exposed area upon imaging.

2.5. *Root Data Collection*

2.5.1. The Profile Wall Method

The profile wall method [21] was used for quantification of root length inside biopores and in bulk soil. Trenches of 2 m-depth were excavated inside the plots with the crop standing and a flat profile wall was prepared towards the inner side of plots. Roots projected on the profile walls were cut to the surface and approximately 0.5 cm-thick of soil was washed away to reveal the protruding roots. A rectangular frame (50 cm × 100 cm: H × W) consisting of grids (5 cm × 5 cm: H × W) was placed onto the prepared wall surface and visual observation on the roots was carried out. Visible roots inside each grid were recorded by visual determination using a root length unit (RLU; 1 RLU = 5 mm), which was then converted to root length (RL, km m^{-2}) and root length density (RLD, cm cm^{-3}). For both post-crop species, five investigations were undertaken every two weeks (Table 2). Roots growing inside and outside biopores were separately recorded and calculated as $RL_{biopore}$ or $RLD_{biopore}$ and RL_{bulk} or $RLD_{biopore}$, respectively. Maximum rooting depth (cm), where 95% of cumulative RL was achieved, was calculated at each time of measurement, following Perkons et al. [10].

Table 2. Root investigation record at the study site in 2013.

Crops	Year	Date *	Growth Stage	DAS ***	Depth (cm) ****
Barley	2013	10 April–11 April	Tillering	188	150
		24 April–25 April	Stem elongation	202	200
		13 May–15 May	Booting	221	200
		27 May–12 June	Anthesis **	235	200
		19 June–24 June	Milk	258	200
Canola	2013	15 April–17 April	Stem elongation	228	200
		26 April–30 April	Flowering	239	200
		21 May–24 May	Development of fruit **	264	200
		12 June–18 June	Ripening I	286	200
		26 June–2 July	Ripening II	300	200

* Dates when the profile wall observation carried out. ** Sampling dates of soil monolith.*** Days after sowing. **** Maximum soil depth investigated during the profile wall observation.

2.5.2. Soil Monolith Sampling

Soil monoliths were taken from the prepared profile wall from soil depths of 45 cm to 155 cm. Three replicated samples with a size of 12 cm × 12 cm × 10 cm containing roots of standing crops were collected at anthesis and development of fruit of barley and canola, respectively. The samples were carefully washed with tap water until the soil was washed away. The remaining parts of the samples were sorted by removing debris and dead roots. The sorted root samples were photo-scanned and the resulted images were analyzed for root length (cm) and root diameter (mm) with the software WinRHIZO Pro (Version 2009c, 32-bit). After the scanning, the roots were transferred to pre-weighed glass vials and oven dried at 65 °C for two consecutive days. Finally, the dry mass of roots was recorded, which was calculated as root biomass (RBM, mg cm^{-3}). Specific root length (SRL, m g^{-1}) was calculated by dividing root length by RBM.

2.5.3. Statistical Analysis

Univariate analysis was performed with the software R [22]. The mixed-effects model [23] of the lmer package [24] was used for further analysis of the data using two factors—pre-crop treatments and post-crop species. Since both measurements (profile wall and soil monolith) consisted of repeated measures, we used the time (two-week interval) and soil depth (10 cm interval) as random variables upon performing the univariate analysis. If required, pairwise *t*-tests ($p \leq 0.05$) and Tukey HSD ($p \leq 0.05$) were carried out across treatments for mean comparisons. Principal component analysis as a multivariate analysis with all the acquired root traits was undertaken using SPSS statistics (ver. 27) in order to classify the effects of the tested factors [20]. Further hierarchical cluster analysis was performed based on the two components generated by PCA using Ward's method with an interval of Euclidean distance. The target number of clusters was set to four, matching the number of crop sequence treatments.

3. Results

3.1. Biopore Size Distribution as Affected by Pre-Cropping

We compared the biopore density of nine size classes at 45 cm of soil depth after growing chicory and tall fescue for two consecutive years. Pairwise *t*-tests comparing the treatments at each biopore diameter classes suggested that only 5–6 mm-sized biopores were significantly affected (Figure 2). The most abundant biopore diameter class was 3–4 mm followed by 4–5 mm, after which biopore density decreased along with the increase in diameter classes.

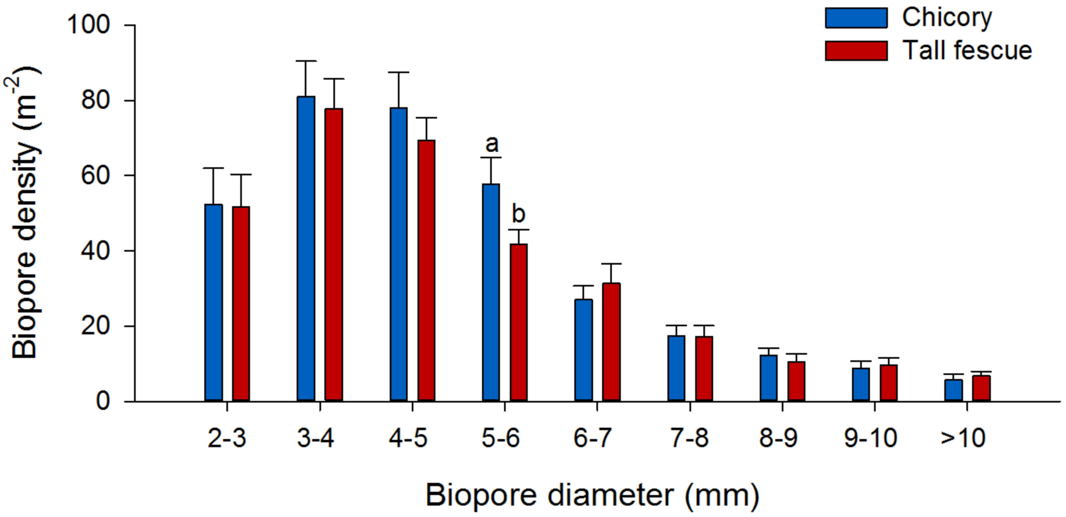

Figure 2. Biopore size distribution from 2–3 mm to >10 mm measured at 45 cm soil depth after growing chicory and tall fescue for two consecutive years. Small letters indicate significant differences between the treatments (pairwise *t*-tests, $p \leq 0.05$).

3.2. Root Growth Inside Biopores and in the Bulk Soil

We compared the root growth of barley at anthesis and canola at flowering inside biopores ($RLD_{biopore}$) and in the bulk soil (RLD_{bulk}) from soil depths of 40–50 cm to 180–190 cm. On average, the $RLD_{biopore}$ of barley after growing chicory and tall fescue were 0.043 and 0.030 cm^{-3}, in which the differences were significant at soil depths of 60–80 cm and 90–100 cm (Figure 3a). The $RLD_{biopore}$ of chicory–canola and tall fescue–canola treatments differed at 50–60 cm soil depth (Figure 3c). The RLD_{bulk} of both barley (Figure 3b) and canola (Figure 3d) was higher when grown after chicory below a soil depth of 80 cm, whereas growing tall fescue as a pre-crop resulted in higher RLD_{bulk} at upper parts of the subsoil (40–80 cm).

To compare the effects of two factors (pre-crop treatment and post-crop species), we averaged the data on the cumulative root length (km m^{-2}) inside biopores ($RL_{biopore}$) and in the bulk soil (RL_{bulk}) between the five measurements. Univariate analysis indicated that the $RL_{biopore}$ was significantly affected by both factors (see Table 3), with the $RL_{biopore}$ of barley and canola higher when grown after chicory compared to tall fescue (Figure 4a). Overall, the $RL_{biopore}$ of canola was significantly higher than that of barley. Significant interactions between the pre-crop treatments and post-crop species for RL_{bulk} were noticed (Table 3). Multiple comparisons showed that the RL_{bulk} of canola was higher when grown after chicory in comparison to that of tall fescue (Figure 4b). Among post-crop species, canola exhibited higher RL_{bulk} compared with barley regardless of pre-crop treatments

Table 3. Univariate analysis on root traits of two winter crops (factor: pre-crop treatment) after growing two fodder crops (factor: Post-crop species).

Root Traits	Pre-Crop Treatment (df = 1)	Post-Crop Species (df = 1)	Interaction (df = 1)
$RL_{biopore}$ *	**6.382 (0.012)**	**59.503 (<0.001)**	2.487 (0.115)
RL_{bulk} *	0.779 (0.314)	**59.026 (<0.001)**	**12.291 (<0.001)**
Maximum rooting depth	**10.729 (0.001)**	**35.500 (<0.001)**	2.805 (0.094)
RBM	**5.825 (0.017)**	**87.725 (<0.001)**	0.012 (0.913)
Root diameter	**203.391 (<0.001)**	**272.701 (<0.001)**	**68.289 (<0.001)**
SRL	**13.746 (<0.001)**	**241.816 (<0.001)**	**11.612 (<0.001)**

* Data were transformed. *F*-values are shown with their probability levels in parentheses. Boldface *p*-values indicate significant effects.

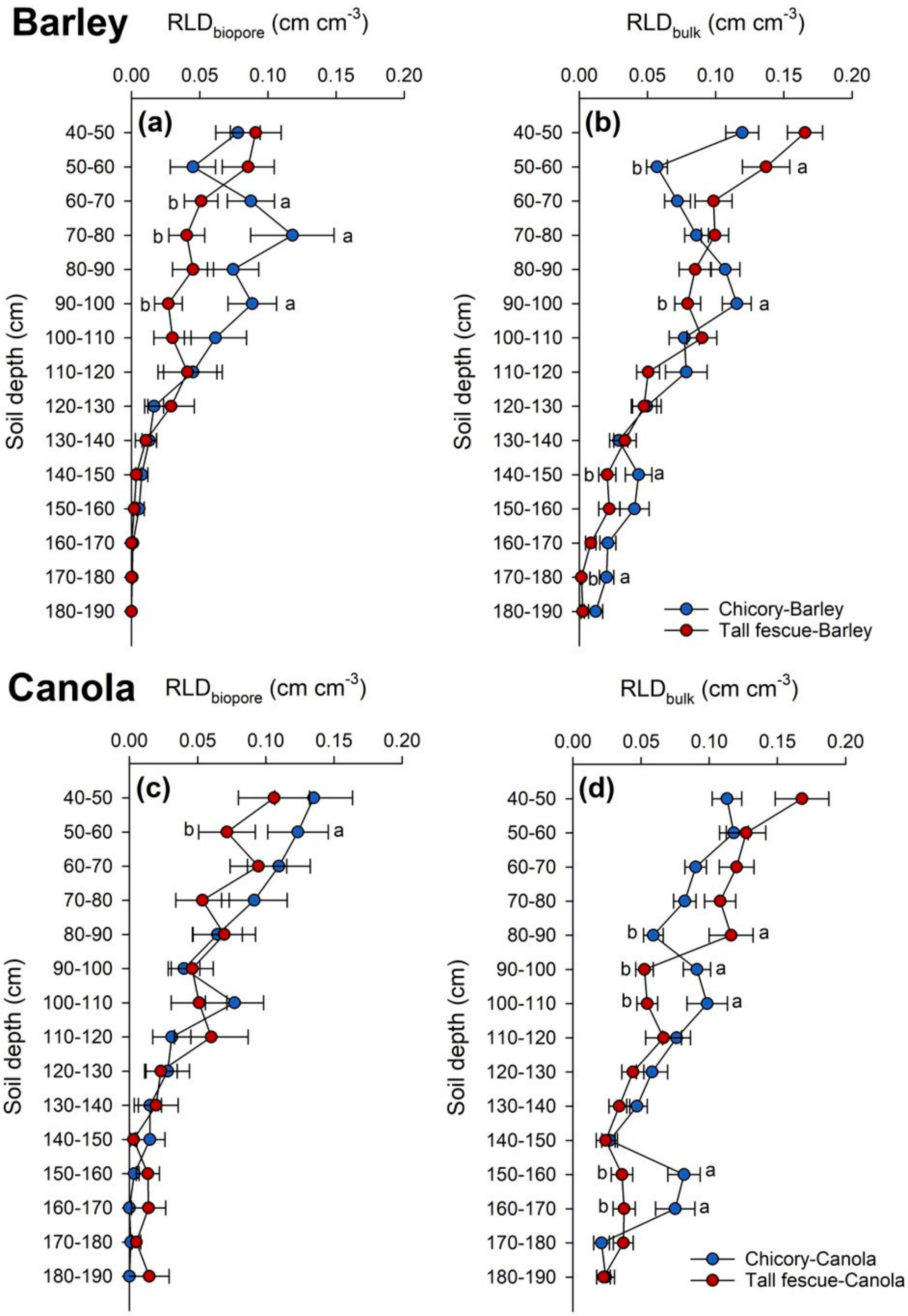

Figure 3. Root length density (RLD, cm cm^{-3}) of barley (**a**,**b**) and canola (**c**,**d**) in the subsoil (40–190 cm) inside biopores (RLD$_{biopore}$; **a**,**c**) and in the bulk soil (RLD$_{bulk}$; **b**,**d**) after growing chicory and tall fescue. Small letters indicate significant differences (pairwise *t*-tests, $p \leq 0.05$).

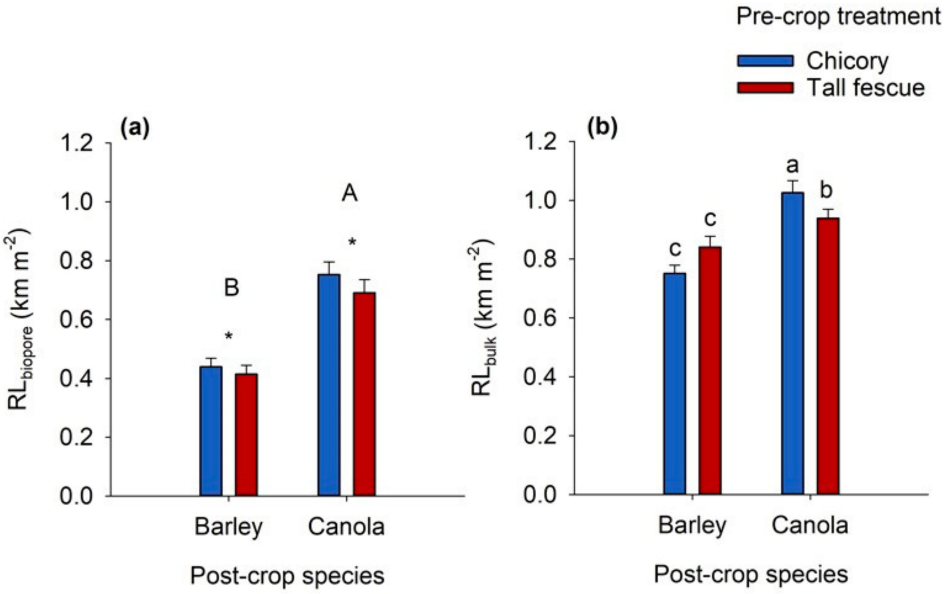

Figure 4. Cumulative root length (km m^{-2}) inside biopores ($RL_{biopore}$, **a**) and in the bulk soil (RL_{bulk}, **b**) measured under 45 cm of soil depth as affected by pre-crop treatments and post-crop species. Mean values (one SE) are shown. Based on the univariate analysis, post-hoc tests were performed for each treatment for $RL_{biopore}$ whereas multiple comparisons were performed across all the treatments for RL_{bulk}. Capital letters and asterisks indicate significant effects of post-crop species and pre-crop treatment, respectively. Small letters indicate significant differences between all the treatments. For results of univariate analysis, see Table 3.

3.3. Root Traits Affected by Crop Sequence

We determined the effects of pre-crop treatments and post-crop species on maximum rooting depth (cm), RBM (mg cm^{-3}), root diameter (mm) and SRL (m g^{-1}). Detailed results from mixed-effects model analysis are shown in Table 3.

Maximum rooting depth was affected by both factors (Table 3). Both winter crops significantly increased maximum rooting depth when grown after chicory compared with growing after tall fescue (Figure 5a). On average, canola deployed significantly deeper roots (109 cm) than barley (99 cm). Similarly, the RBM of both winter crops was significantly higher when grown after chicory compared with tall fescue (Figure 5b). Across the pre-crop treatments, barley produced higher RBM in comparison to canola.

Root morphological traits (root diameter and SRL) showed significant effects from both factors; however, their interactions were found to be significant (Table 3). The root diameter of barley grown after chicory was significantly larger compared with barley grown after tall fescue (Figure 5c). Similarly, when canola was grown after chicory, root diameter increased compared to canola grown after tall fescue. Within each pre-crop treatment barley exhibited larger root diameter than canola.

Comparisons of SRL of barley between pre-crop treatments resulted in significant differences, in which the SRL of barley grown after tall fescue was significantly higher compared with barley grown after chicory (Figure 5d). No pre-crop effects on SRL were found for canola. The SRL of canola was significantly higher than barley regardless of pre-crop treatments.

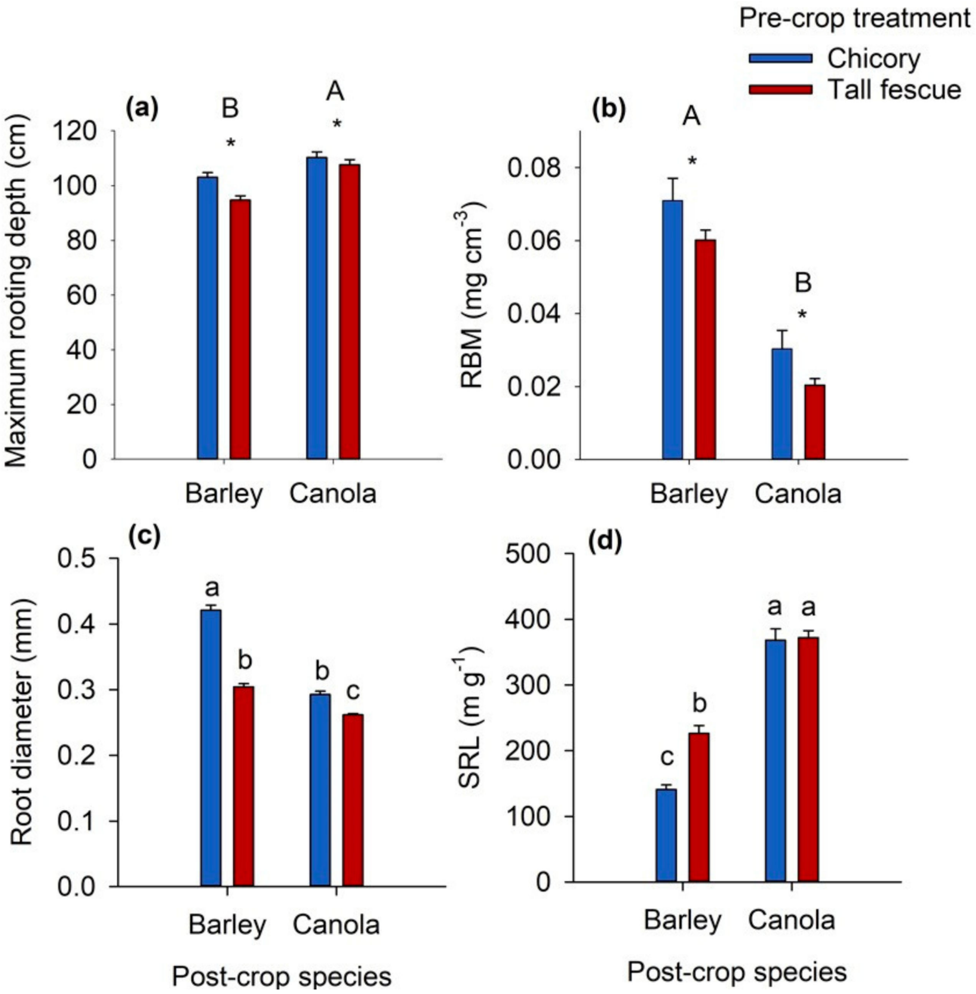

Figure 5. Maximum rooting depth (cm, **a**), root biomass (RBM, mg cm^{-3}, **b**), root diameter (mm, **c**) Specific root length (SRL, m g^{-1}, **d**) measured under 45 cm of soil depth as affected by crop sequence in 2013. Capital letters and asterisks indicate significant effects of post-crop species and pre-crop treatment, respectively. Small letters indicate significant differences between all the treatments. Mean values (one SE) are shown. For results of univariate analysis, see Table 3.

3.4. Principal Component Analysis

Principal component analysis with root traits of barley and canola—RL$_{\text{biopore}}$ (km m^{-2}) and RL$_{\text{bulk}}$ (km m^{-2}), maximum rooting depth (cm), RBM (mg cm^{-3}), root diameter (mm), SRL (m g^{-1}) revealed two main components (PC1 and PC2; Figure 6a,b). The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was 0.768 and Bartlett's test of sphericity indicated that the tested variables were orthogonal (unrelated to each other) with significance ($p \leq 0.001$).

PC1 and PC2 explained the variances of 66 and 15%, respectively. A distinguished grouping of objects between the post-crop species (Figure 6a) is shown along PC1, which captured all root traits measured (Figure 6b). Chicory and tall fescue objects were grouped along PC2 (Figure 6a) with root traits such as maximum rooting depth and root diameter (Figure 6b).

Further hierarchical clustering based on the two components was performed to generate four clusters matching the number of crop sequence treatments. Clusters 1 and 2 consisted of crop sequence objects mainly from barley grown after chicory (except one object grown after tall fescue). Cluster 3 included the objects from canola (three after chicory and one after tall fescue). The majority of objects in cluster 4 were from crop sequences that involved chicory as a pre-crop (10 out of 13) among which the post-crop species were equally distributed (five barley and five canola objects).

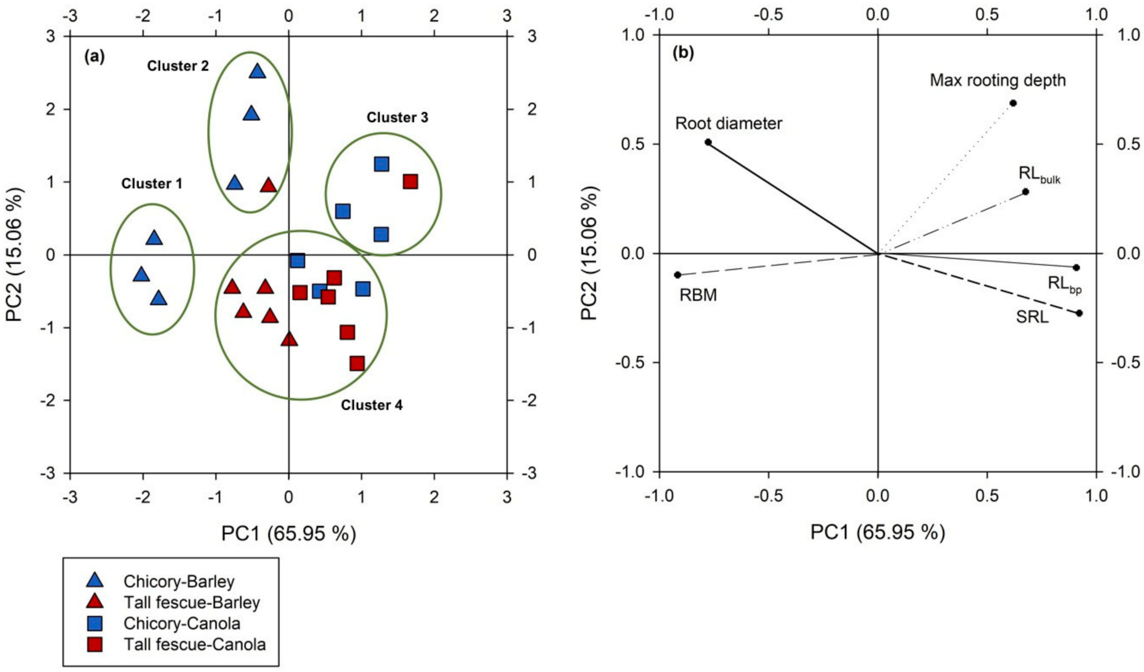

Figure 6. Biplots showing location of the single objects (**a**) and trait vectors (**b**) based on principal component analysis on $RL_{biopore}$, RL_{bulk} (km m^{-2}), maximum rooting depth (cm), RBM (mg cm^{-3}), root diameter (mm) and SRL (m g^{-1}) measured under 45 cm of soil depth with four crop sequence treatments. Clusters were formed based on hierarchical cluster analysis.

4. Discussion

4.1. Contrasting Root Traits between Barley and Canola

Both univariate and multivariate analyses indicated that the root traits of two winter crops differed greatly. Significantly larger root diameter of barley compared with canola under 45 cm of soil depth is in accordance with previous studies [25] that reported on the larger root diameter of cereals compared with summer oilseed crops in deeper soil layers. The higher SRL of canola can be related to its smaller root diameter. This is in contradiction to the general assumptions stating that the SRL of dicots is generally lower [26]. However, such root morphological traits are also affected by plant age and soil conditions [27] and nutrient and water availability [28]. The higher SRL of crop plants might be beneficial since it increases the absorptive surface area of roots for resource acquisition [29], which can however also involve substantial costs for root construction [30]. The higher RBM of canola compared with barley is not in accordance with previous findings [25], which can be attributed to the smaller root size of canola. Lower rooting density of canola in comparison to barley, oats and ryegrass was previously reported by Eutric Cambisol [25]. The distinction in root traits between barley and canola suggests that they have different strategies for root growth owing to their contrasting root system architecture [31]. While barley as a monocot tends to increase rooting density per unit volume of soil, the dicots have more efficient resource uptake per unit root length [12].

4.2. Consistent Response towards Increased Biopore Density

Our results on the distribution of biopore density indicate that the taprooted chicory increased the density of large-sized biopores. Although similar results were shown previously [8,10], our new approach using image analysis to determine the diameter of each biopore revealed that the effects were restricted to a specific size-class (5–6 mm). It is clear that the larger root diameter of chicory compared with tall fescue [32] was capable of creating the large-sized biopores due to high penetration capacity [14].

The RLD of both winter crops was affected by elevated biopore density; however, the effects varied along the soil profile. The detailed observations on rooting density at 10 cm-intervals revealed that the higher biopore density led to increased root lengths inside biopores for both crops consistently at the upper subsoil layers (<100 cm). On the other hand, biopore effects were shown for root length in bulk soil at deeper soil layers—below 100 cm of soil depth. Such preferential root growth via increased soil biopores and subsequent increase in rooting density in bulk soil has been reported previously [10].

When the root data were averaged over the growing season, the $RL_{biopore}$ and maximum rooting depth of both crops were higher under elevated biopore density suggesting that the root deployment in the subsoil was enhanced with the increase in biopore density. This indicates that both species preferred to grow into the area with less penetration resistance [33,34].

4.3. Contrasting Response towards Increased Biopore Density

We also observed contrasting responses from barley and canola towards the biopore density alteration. In the case of barley, the cumulative root length in the bulk soil did not show significant differences between the pre-crop treatments, whereas canola did exhibit effects. The inconsistency in biopore effects was also shown with the morphological root traits (e.g., SRL). Despite the evidence of biopore effects shown with the depth-wise analysis on rooting density of barley in bulk soil (RLD_{bulk}), the cumulative root length averaged over the season did not reveal the same effects. This indicates that biopore effects in the subsoil can vary over the season and in different soil depths. In fact, at the same study site, the effects of elevated biopore density on subsoil root growth were shown in the early season in the case of spring wheat [9]. Testing the same effects on barley and canola also revealed depth-wise variation—increased rooting density in bulk soil only at deeper soil layers [10]. Therefore, our results do not necessarily contradict previous findings but rather indicate the inconsistency of biopore effects at different temporal and depth scales. The important implication of our study is that effects of elevated biopore density differ according to the choice of the following crop species, which has not been reported so far and has an important implication for crop sequence management.

We are cautious to conclude on the contrasting response shown by barley and canola by looking at the results on root diameter as the effects were rather similar, yet the magnitude differed. While both crops responded similarly by increasing the root diameter under high biopore density, the degree of increase was more pronounced for barley than canola. For barley, we have previously reported that there was a morphological shift towards larger root size when the crop was grown with abundant biopore density [16]. Such a phenomenon might be attributed to, firstly, radial swelling of roots inside biopores due to unrestricted radial pressure in large-sized pores [17]. Considering that both crops showed a similar increase in rooting density inside biopores and root size, the difference in the degree of root thickening might have occurred due to their root architectural differences inside biopores. Athmann et al. [13] observed that barley roots were mainly in direct contact with the walls inside biopores, whereas canola produced more lateral roots which were in contact with the pore walls. This means that the proportion of roots undergoing radial swelling might have been higher for barley roots than canola roots, leading to a higher root diameter of the former.

It is also possible that the axial pressure exerted during root elongation through the pore walls upon re-entry [35] might have occurred more frequently for barley roots than canola roots, considering their architecture formed inside the biopores, as mentioned previously. The resulting larger root size might further facilitate rapid root growth in the bulk subsoil [14] but also might demand higher costs for construction and maintenance compared with small-sized roots and assuming the same root densities.

The SRL of barley exhibited the effects of biopore density alteration whereas the SRL of canola did not differ between the pre-crop treatments. The decrease in SRL for both winter crops with an increase in biopore density should have been led by an increase in root diameter [30]. This also indirectly implies that the requirement for excessive root surface area was diminished with an increase in biopore volume [18]. In general, a decrease in SRL is not considered to be a positive reaction of plants that maximizes soil contact for efficient nutrient uptake [36]. In fact, smaller SRL can be considered to provide less favorable geometric conditions of diffusion to the root surface because delivering soil volume is proportional to reciprocal root radius [37]. However, the actual efficiency of root systems for efficient nutrient uptake also depends on root hair geometry and mycorrhiza, which were not quantified in this study. Thus, SRL alone cannot be used as an indicator of nutrient uptake efficiency.

4.4. Classification of Biopore Effects

Interpretation of the results derived from the generic univariate analysis is rather complex due to the temporal (time of observation), spatial (soil depth), phenotypic (root traits) and genotypic (crop species) variation. Bodner et al. [20] previously made claims about the disadvantages of generic univariate analysis on a single root trait. First of all, root traits are not independent from each other but rather are linked to another, hence interact [38]. Moreover, more than a single trait is required to properly address the functionality and adaptation of root systems. Finally, outcomes of single trait-based comparison are often research-specific, leading to results contradicting each other.

Based on our PCA and hierarchical cluster analysis, roots' responses to altered biopore density revealed a tendency to be species specific. While pre-crop treatments were clearly divided between clusters 1, 2 (chicory, except for one object) and 3 (tall fescue), canola objects were not clearly divided based on the pre-crop treatments. The cluster location and vector distance in the biplots indicate that pre-crop effects on barley roots were classified due to the both density-based and morphological root traits explained by PC1 and PC2, respectively. This should be related to the increase in rooting density of large root diameter classes in biopore-rich soil conditions at the study site [16]. The lesser degree of clustering among canola objects for pre-crop treatments should have been derived from its morphological traits, which did not reveal pre-crop effects. Distribution of objects was classified more distinctively based on the root traits of post-crop species rather than those of pre-crop treatments, except for cluster 4, for which the distribution should be attributed to RBM and maximum rooting depth, as shown with the vector plot. The distinctive clustering between barley and canola objects was also due to their differences in root diameter, for which the winter cereal resulted in larger root size than the oilseed crop, which might be due to the smaller proportion of large roots of canola owing to its higher branching capacity as laterals [25].

4.5. Biopore Utilization in Arable Fields

There have been long debates on the pros and cons of roots growing inside soil biopores. When the advantages of such phenomenon are considered, researchers note that it provides crop plants with physically easier access to the subsoil, which helps in better soil resource acquisition [33,39]. More importantly, according to Pierret et al. [40], in comparison to the bulk soil, the adjacent zone around the soil macropore, the so called macropore sheath (MPS), can be described as more biologically active with more major (N and P) and minor nutrient (Fe and Mn) contents that can be utilized by crop plants. In contrast, when roots are not in contact with pore walls, it is possible that the roots are trapped inside the channels, which results in root clumping [41–43]. However, recent studies have found that such crop roots were not trapped inside the soil channels but that re-entry of them into the bulk soil through pore walls occurred [13,44]. In light of the contradictory findings on biopore effects, it might be useful to refer to the approach of Stirzaker et al. [33] who defined the ability of roots to utilize biopores through root morphological traits—root diameter in this case. For example, the study described how only roots larger than 0.8 mm in diameter were able to penetrate the pore wall, indicating that the re-entry capacity might depend on the morphological traits of the standing crop species.

Species-specific response towards biopore density alteration might be one of factors to be considered when planning cropping sequences. In fact, topsoil nutrient retention with N-fixing legumes [45,46] or timely capturing of the leaching-prone nitrate in subsoil layers [47] has been a top priority in consideration of crop rotation management. However, according to our results, it is possible that root plasticity in response to biopore formation and its variation between crop species might be worth considering for crop sequencing and its resulting nutrient acquisition potential from arable subsoil. This perspective is more important for agricultural systems with fewer outsourced farm resources, e.g., organic agriculture [48,49]. In the future, inclusion of greater number of species, varieties and genotypes [50,51] would provide a more concrete view on the functional classification of root plasticity, which would enhance breeding program formulation for biopore-induced root traits. To do so, root architectural [52] and anatomical [53] traits should be considered as part of an integrative phenotyping approach.

5. Conclusions

Our hypotheses are accepted since the results show that (a) both plant species responded to the altered biopore density as affected by the crop sequence, in which (b) two winter crops with contrasting root systems differed in response according to their (c) morphological traits rather than root length inside and outside of biopores. We conclude that large-sized biopores induce deeper rooting of winter crops that might increase soil resource acquisition potential, which is considered to be more important for agricultural systems with less outsourced farm resources, e.g., Organic Agriculture. Crops with contrasting root systems respond differently to biopore alteration in terms of root morphology, which should be taken into account upon exploiting biopore-rich conditions in arable fields. The species-specific variation suggests the needs for further detailed research with a greater number of species, varieties and genotypes for functional classification of root plasticity.

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