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ORIGINAL PAPER

Root growth dynamics inside and outside of soil biopores as affected by crop sequence determined with the profile wall method

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Abstract Taprooting crop species are capable of creating soil biopores (>2 mm in diameter) in the subsoil due to their large root size and deep-rooting habit. The aim of this study was to quantify root growth dynamics of wheat in the subsoil during its complete growth season as affected by crop sequence. Temporal observation on root length (km m^{-2}) of wheat inside and outside of biopores at four growth stages (tillering, booting, anthesis, and milk) was conducted by using the profile wall method under the two crop sequence treatments involving two precrops, viz., chicory and tall fescue. Frequency of biopore presence measured on vertical profile walls depended on the choice of precrops in which chicory precrop resulted in higher frequency (2.3 %) compared with tall fescue (1.5 %). Root length of wheat measured inside biopores was significantly higher when grown after chicory (0.024 km m^{-2}) in comparison to tall fescue (0.006 km m^{-2}). On average, root length outside biopores after growing chicory was 45.9 % higher than tall fescue until the stage of anthesis. We conclude that at the site under study biopores as pathways for rapid root growth into deeper soil layers allow roots to re-enter and explore the subsoil. Thus, cereals cultivated in rotation with taprooted crops can draw benefit from enhanced uptake of water and nutrients from deeper soil layers during early growth stages. Model simulations with various abiotic and biotic factors will be helpful to reveal the direct evidence of biopore-root-shoot relationship in the future.

Keywords Soil biopore creation · Root length · Wheat · Fodder cropping · Subsoil

Introduction

Large taproots can penetrate the compact subsoil (Materechera et al. 1992), create and leave round-shaped void channels (McCallum et al. 2004; Han et al. 2015), otherwise called biopores (>2 mm in diameter; Kautz 2014), after the root materials are decayed (Jones et al. 2014; Kautz et al. 2014). Biopores can be used as preferential pathways for root growth (Valentine et al. 2012; Kautz 2014) and give plants access to the subsoil resources (Kautz et al. 2013a). With an image-based analysis of pore-roots relationship, Stewart et al. (1999) found preferential location of grass roots adjacent to soil pores on two Vertisols. Similar results were found on Brown Lowland soil, Pseudogley and Ordinary Andosol (Hatano et al. 1988) in which distribution of maize roots significantly depended on presence of soil macropores.

In a recent review, Lynch and Wojciechowski (2015) have clearly stated the benefits of deep-rooting of crop plants into the subsoil (e.g., water uptake, N capture, and C sequestration). Nutrient present in the subsoil (soil beneath tilled horizon) might contribute up to two thirds of total demand of crop plants (Kautz et al. 2013a). Eighteen to 38 % of mineral N uptake by winter wheat (Kuhlmann et al. 1989), 30–85 % of total P uptake (Fleige et al. 1981; Kuhlmann and Baumgärtel 1991), and 34 % of K uptake by spring wheat (Kuhlmann

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1990) from the subsoil were previously reported. Kirkegaard et al. (2007) also found an additional increase in grain yield of spring wheat of 620 kg ha^{-1} when supplied with 10.5 mm of subsoil water under moderate post-anthesis stress on a red Kandosol.

Root elongation to the deeper soil layers is, however, often restricted with high mechanical resistance in the soil, and overall plant growth is affected as a result (Valentine et al. 2012; Lynch and Wojciechowski 2015). When wheat was grown on compact soil of deep loamy sand, overall root length was 66 % shorter than those in loosen soil, and shoot N and K contents were reduced by 12–14 % (Atwell 1990). On the other hand, a commercial wheat cultivar, Wyalkatchem, increased root length and root mass by 36 and 24 %, respectively, in response to deep-ripping of the compact layers of sandy soil resulting in 19 % increase in grain yield (Chen et al. 2014). Thus, provision of pathways for roots by promoting large sized biopore formation with crop rotation management is considered to be beneficial (Kautz 2014), especially in regions where crop plants suffer from high mechanical impedance.

Previous studies (e.g., Kautz et al. 2013b; Perkons et al. 2014) revealed that root length in biopores and the share of roots growing through biopore channels in the subsoil may vary considerably over time and along the soil depth. Recently, a novel visual approach has also shown that roots elongating inside biopores cross the pore walls in deep soil horizons (Athmann et al. 2013), thus can re-explore the bulk soil. Improved water (Gaiser et al. 2012) and N uptake (Volkmar 1996) by wheat as a function of pore-assisted root growth suggest that plants are capable of utilizing subsoil resources when accessible (Köpke et al. 2015). Increased plant P availability (Barej et al. 2014) and microbial activity (Kuzyakov et al. 2007; Uksa et al. 2014) in zones adjacent to large sized biopores also indicate that the biologically created pores might be hotspots for nutrient acquisition (Kautz et al. 2013a).

Despite the growing evidence, however, it still remains unclear how increased biopore presence caused by cultivation of taprooted crops may influence the temporal dynamics of root growth of subsequent crops. Only few studies have attempted to include “time” as an important factor to determine biopore-root association (e.g., Kautz et al. 2013b). Considering that nutrient requirements of crop plants differ between growth stages (Peng et al. 2012; Girma et al. 2014), quantification of temporal variation in biopore effects on root growth is essential for understanding the relationship between belowground and aboveground production within a cropping cycle (Neukirchen et al. 1999).

Moreover, root growth in association with biopore formation with field crops is often restricted to qualitative approaches (Bengough 2003) due to methodological difficulties (Hutchings and John 2003). Previous studies (e.g., Ehlers et al. 1983; Kautz et al. 2013b; Perkons et al. 2014) have proven that the profile wall method (Böhm 1979) can be adopted for observation of temporal root growth with effective visualization of roots in association with soil biopores along the deep soil horizons.

The aim of this study was to determine root length of wheat inside and outside of biopores as influenced by (i) previously cultivated fodder crop with or without the capability to create large sized biopores and (ii) the crop growth stage. We hypothesized that (a) root growth inside biopores is more pronounced with a crop sequence including a taproot system than a fibrous root system, and that (b) a crop sequence including taprooted crops favors a rapid root development of following crops in the subsoil leading to higher rooting density, especially during the vegetative phase of crop development.

Materials and methods

Experimental site

The field experiment was performed from 2010 to 2012 at the Campus Klein-Altendorf research station (50° 37′ 9″ N, 6° 59′ 29″ E) in Rheinbach, Germany. The soil was classified as Haplic Luvisol (hypereutric, siltic) derived from loess (IUSS Working Group WRB 2006). Vetterlein et al. (2013) described six distinct horizons at the study site, namely Ap (0–31 cm), A1/Bt (31–42 cm), Bt1 (42–63 cm), Bt2 (63–86 cm), Bwt (86–116 cm), and 1eCw (116+cm). The mean annual precipitation and temperature at the study site recorded from 1956 to 2010 were 603.4 mm and 9.4 °C. Figure 1 shows monthly recorded weather data in 2012. Highest precipitation and air/soil temperature were recorded in October and August, respectively.

Field experiment

The investigations (2010–2012) consisted of two distinctive phases. During the first phase (2010–2011) of the experiment, two fodder crop species, viz., chicory (*Cichorium intybus* L. “Puna”) with a taproot system and tall fescue (*Festuca arundinacea* Schreb. “Hykor”) having a fibrous root system were grown for two consecutive years as precrops in four replicated plots. In 2012, wheat (*Triticum aestivum* L. “Scirocco”) was grown as a following annual field crop where the precrops were cultivated.

The precrops were sown in April 2010. Sowing density of chicory and tall fescue were 5 and 30 kg ha^{-1}, respectively. Both precrops were mowed during summer seasons for three and five times in 2010 and 2011. Shoot residues were left homogenously broadcasted on soil surface. No additional fertilizer was given to the precrops. Prior to the sowing of wheat, the mulched residues were inverted by mold board plowing

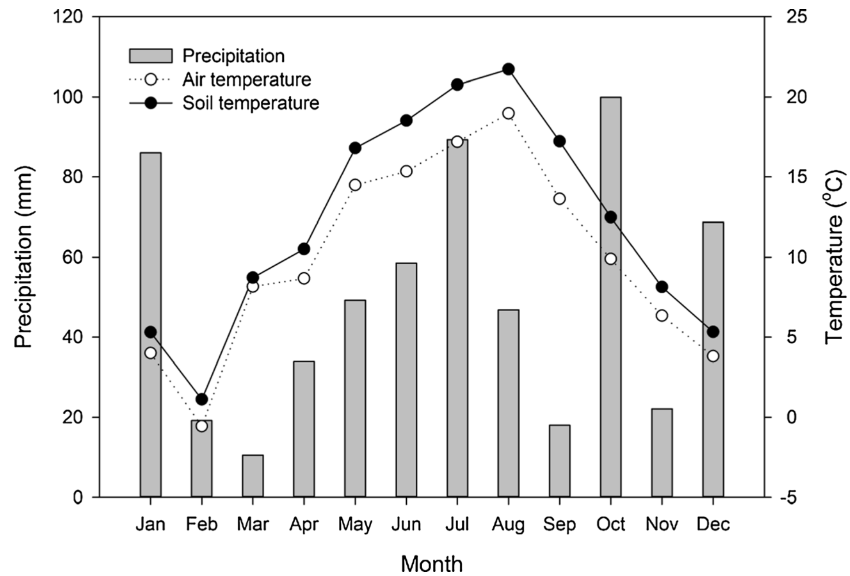

Fig. 1 Precipitation, air and soil temperature (at 20 cm of soil depth) at Campus Klein-Altendorf, Rheinbach, Germany in 2012

(30-cm soil depth). Wheat was sown in March 26 in 2012 with a sowing density of 400 grains m^{-2} and a row width of 12 cm, fertilized with 40 kg N ha^{-1} (calcium ammonium nitrate) and harvested on August 9, 2012. The single plot size was 6× 10 m.

Sampling

Soil physical and chemical properties

Undisturbed soil core samples (54 mm Ø, 40 mm height) with seven replicates were collected with a manual auger in April 2012 at soil depths of 0–15, 15–45, 45–60, and 60–75 cm. Pore volume (%), air capacity (%), and bulk density (g cm^{-3}) were determined by desorptive water retention curve generation. Detailed description for analysis of soil physical property is available in Perkons et al. (2014). Using a Pürckhauer auger, two replicated soil samples were collected for three times from May to July 2012 at the soil depth of 45–105 cm for analysis of soil mineralized N (Table 1). Skalar Continuous Flow Analyzer was used for mineral N concentration (NO_3^- and NH_4^+) analysis (kg ha^{-1}; soil N_{min} concentration).

Monolith sampling

Four replicated soil monoliths (25×10×10 cm) containing the roots of chicory and tall fescue were collected at 45–105 cm of soil depth in 2011. The collected samples were stored in the refrigerator till washing with tap water in order to remove the non-root materials from the samples. The washed roots were further sorted for debris removal. The sorted root samples were photo-scanned (Epson Perfection V700), and the resulted images were analyzed with “WinRHIZO Pro” (version 2009c, 32 Bit).

Profile wall method

The profile wall method was used to estimate rooting depth (cm) of chicory and tall fescue in 2011 and root length (km m^{-2}) of wheat inside biopores and in the bulk soil in 2012. Trenches (2.5×2×2 m) were formed by using a bucket excavator inside the wheat plot area. A straight vertical wall was prepared by flattening the surface to the maximum depth of 2 m. Roots exposed at the profile wall were removed with scissors. Surface of vertical walls was jet-sprayed with water (300 kPa) removing a layer of approximately 0.5-cm thickness and exposing the roots. A rectangular frame (0.5×1 m) consisting of identical grids (5×5 cm) was attached to the wall for counting root-length units equivalent to 5 mm of visible root length in each grid. Root-length units were converted to root length (km m^{-2}). In this paper, root length was calculated with the data acquired beneath 45-cm soil depth only. For the precrops, one observation was made in 2011, whereas in 2012, four observations were made with wheat at intervals of 2 weeks (Table 1). Growth stages were recorded based on Lancashire et al. (1991). At each time of observation, presence of biopore (>2 mm) inside each grids was recorded and calculated as frequency of biopore presence (%).

Plant shoot growth

Four replicated shoot biomass samples of precrops from 2010 to 2011 and of wheat in 2012 were collected from the area of 0.5×0.5-m size (Table 1). Shoot growth parameters, viz., chlorophyll content in plant leaves (SPAD value), leaf area index (LAI), and plant height (cm) were measured at each time of sampling. Shoot biomass was measured as dry matter (t ha^{-1}) with oven drying (105 °C) from which shoot N and P/

Table 1 Dates of root investigation, soil sampling, and shoot biomass harvest during four growth stages of wheat in 2012

Growth stage	Root investigation	Soil sampling[a]	Shoot biomass harvest
Tillering	7 May–8 May	3 May	16 May
Booting	21 May–23 May	–	30 May
Anthesis	5 Jun–22 Jun	5 Jun	12 Jun
Milk	22 Jun–5 Jul	16 Jul	25 Jun

[a] Samples for analysis on soil mineral N; sampling for soil physical properties was done on 11–12 April

K contents were measured using the Dumas method and atomic absorption spectrometry (AAS), respectively.

Statistical analysis

Statistical analysis of data was done with the R package (R Core Team 2014). Data were tested for their normal distribution (Shapiro-Wilk test, $P \leq 0.05$) prior to any further statistical tests. Based on that, the root data were required to be transformed. For further univariate analysis, linear mixed-effects model (Pinheiro and Bates 2000) was used. If required, post hoc tests (Tukey's HSD, $P \leq 0.05$) or *t* tests ($P \leq 0.05$) were carried out.

Results

Root and shoot growth of precrops

Maximum rooting depths (≥95 % of root length) of chicory and tall fescue in 2011 reached 180 and 130 cm of soil depth, respectively (Table 2). Across the soil depth, mean diameter of chicory roots (0.43 mm) was larger compared with tall fescue roots (0.30 mm; Table 3). In contrast to root diameter, the average root-length density (cm cm^{-3}) of tall fescue (2.46 cm cm^{-3}) at 45–105 cm of soil depth was higher compared with chicory (1.56 cm cm^{-3}).

Accumulative amount of biomass, N, P, and K mulched with the shoot materials of chicory and tall fescue in 2010 and in 2011 are shown in Table 4. Grand total value showed that the amount of N, P, and K mulched was significantly higher for chicory in comparison to tall fescue (*t* tests; $P \leq 0.05$).

Soil physical properties

Frequency of biopore presence in the subsoil depended on the choice of precrops (Pearson chi-squared test; $P \leq 0.05$; Fig. 2). Overall frequency of biopore presence was 2.3 % after taprooted chicory and 1.5 % after fibrous-rooted tall fescue. Soil air capacity after growing tall fescue (9.7 %) at the soil depth of 45–60 cm was higher compared with chicory (4.0 %; Fig. 3b), whereas pore volume (Fig. 3a) and bulk density (Fig. 3c) between the two treatments did not reveal any significant differences.

Temporal root growth

Root length of wheat in the subsoil (>45 cm) inside biopores was significantly influenced by crop sequence as well as growth stage (Table 5). When grown after chicory precrop, wheat had higher root length inside biopores (0.024 km m^{-2}) compared with tall fescue-wheat sequence (0.006 km m^{-2}; Fig. 4a). The highest root length inside the pore channels was shown at the growth stage of milk (0.026 km m^{-2}) followed by anthesis (0.020 km m^{-2}), booting (0.015 km m^{-2}), and tillering (≤0.001 km m^{-2}) regardless of crop sequence.

Effects of crop sequence on root length outside biopores of wheat varied over the growth stages (Table 5). Comparisons between the two crop sequence treatments at each stage of

Table 2 Cumulative share (%) of root length of chicory and tall fescue at 0–200 cm of soil depth in 2011

Soil depth (cm)	Chicory (%)	Tall fescue (%)
0–10	10.72	26.86
0–20	20.30	43.27
0–30	29.88	55.86
0–40	37.12	64.64
0–50	42.84	70.94
0–60	48.01	75.69
0–70	53.51	79.76
0–80	58.44	83.60
0–90	63.46	86.46
0–100	68.40	89.29
0–110	72.17	91.73
0–120	75.68	94.19
0–130	79.66	95.71[a]
0–140	83.45	97.03
0–150	87.11	98.05
0–160	90.57	98.81
0–170	93.65	99.40
0–180	96.59[a]	99.83
0–190	98.44	99.94
0–200	100.00	100.00

[a] Maximum rooting depth

Table 3 Root diameter (mm) and root-length density (cm cm^{-3}) of chicory and tall fescue measured at 45–105 cm of soil depth in 2011

Soil depth (cm)	Mean root diameter		Root-length density	
	Chicory	Tall fescue	Chicory	Tall fescue
45–55	0.459±0.023 a	0.310±0.004 b	1.980±0.318 b	3.196±0.184 a
55–65	0.428±0.017 a	0.303±0.008 b	1.882±0.425	2.587±0.216
65–75	0.422±0.014 a	0.306±0.008 b	1.278±0.221 b	2.262±0.139 a
75–85	0.422±0.010 a	0.291±0.007 b	1.270±0.092 b	2.380±0.279 a
85–95	0.420±0.008 a	0.285±0.004 b	1.211±0.322 b	2.502±0.351 a
95–105	0.451±0.007 a	0.289±0.004 b	1.722±0.620	1.806±0.223

Different letters indicate significant differences (*t* test; $P \leq 0.05$). Data were transformed for analysis, but mean and SE are shown

investigation revealed that chicory-wheat sequence resulted in higher root length outside biopores than tall fescue-wheat sequence at the stages of tillering, booting, and anthesis (Fig. 4b).

Ratio of root length inside and outside biopores of chicory-wheat and tall fescue-wheat was 0.096 and 0.031, respectively. Univariate analysis, however, revealed only an influence of growth stage (Table 5) in which the highest ratio was shown at anthesis (0.048).

Dynamics of shoot growth and soil N_{min} concentration

SPAD value, LAI, plant height, shoot biomass (Fig. 5a), N (Fig. 5b), and K uptake (Fig. 5d) of wheat did not reveal any significant effects of crop sequence but of growth stage (Table 5). P uptake by shoot was significantly affected by crop sequence and growth stage (Fig. 5c). Final yield parameters did not reveal any significant differences between the crop sequence treatments (Table 6). Soil N_{min} concentration measured at 45–105 cm of soil depth revealed no difference between the crop sequence treatments but showed substantial decrease from tillering (73.7 kg ha^{-1}) to anthesis (24.9 kg ha^{-1}) and slight increase at the time of milk (32.7 kg ha^{-1}; Fig. 6).

Discussion

Our hypotheses that a crop sequence with a precrop taproot system (a) increases root growth of following crops inside biopores and (b) favors the rooting of subsequent crops, especially during the vegetative stages of crop development, were confirmed. As previously reported, deep-rooting capacity and bigger root size affected formation of large sized pores (Materechera et al. 1992; McCallum et al. 2004; Han et al. 2015) resulting in higher frequency of biopore presence after cultivation of the taprooted preceding crop. Overall frequency of biopore presence in vertical soil profile at the study site is in

Table 4 Plant biomass, N, P, and K uptake during chicory and tall fescue cultivation from 2010 to 2011

Date	N	Biomass (t ha^{-1})		N (kg ha^{-1})		P (kg ha^{-1})		K (kg ha^{-1})	
		Chicory	Tall fescue	Chicory	Tall fescue	Chicory	Tall fescue	Chicory	Tall fescue
7 Jul 2010	4	3.60 a	2.38 b	80.62 a	53.51 b	10.78 a	6.62 b	236.30 a	93.72 b
20 Aug 2010	4	3.09 a	1.97 b	59.09 a	41.77 b	8.97 a	5.74 b	190.51 a	65.24 b
17 Nov 2010	4	1.62	2.22	27.48 a	43.29 b	5.44	6.62	74.86	68.69
Total	4	8.32 a	6.57 b	167.19 a	138.58 b	25.20 a	18.99 b	501.67 a	227.65 b
11 May 2011	4	3.08 b	5.03 a	67.23	59.13	16.75	11.25	205.56	127.70
30 Jun 2011	4	3.24 a	1.17 b	42.52	17.73	12.89 a	4.01 b	135.97 a	31.83 b
11 Aug 2011	4	1.88	1.02	33.37	18.12	8.01	3.66	91.88 a	27.79 b
12 Sep 2011	4	1.18	0.96	26.71	18.07	5.95	4.19	64.74	45.44
3 Nov 2011	4	0.90	0.64	22.12 a	9.31 b	3.38	2.22	47.50 a	12.14 b
Total	4	10.29	8.82	191.95	122.36	46.99	25.32	545.66	244.90
Grand total	4	18.60	15.39	359.15 a	260.94 b	72.18 a	44.31 b	1047.33 a	472.55 b

Different letters indicate significant differences between treatments at each date (chicory vs tall fescue; *t* tests, $P \leq 0.05$)

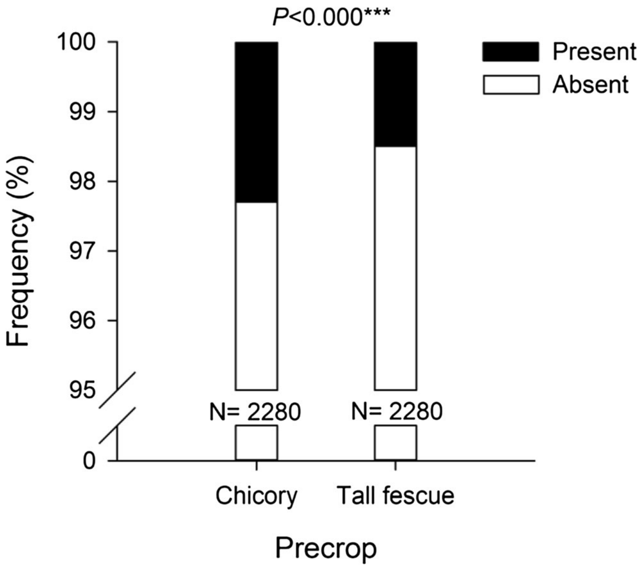

Fig. 2 Frequency of biopore presence appearing in 5 × 5-cm grids on the profile wall beneath 45-cm soil depth during four times observation in 2012. ***Pearson chi-squared test indicates significant relationship between crop sequence and biopore presence

agreement with previous findings (e.g., Ehlers 1975; Wuest 2001) that also reported on low proportion of biopore volume to total soil volume (e.g., >0.2 %) as a function of tillage. Higher rooting density of tall fescue precrop in comparison to chicory precrop, especially in the upper part of the subsoil, is in accordance with previous studies that found an extensive rooting habit of the *Festuca* species (White and Scott 1991; Carrow 1996; Huang and Gao 2000).

Significant effects of crop sequence on root length of wheat inside biopores indicate a strong relationship between increased biopore presence and preferential root growth via biopore channels (Hatano et al. 1988; Stewart et al. 1999; Valentine et al. 2012; Kautz 2014). More pronounced root growth in the bulk soil when grown after chicory during the vegetative stages might have been derived from the early penetrated roots inside biopores that, presumably, have re-entered the bulk soil (Athmann et al. 2013). The resulted intensive root-soil contact might enhance mobilization potential in the subsoil (Veen et al. 1992; Jungk and Claassen 1997). Also, the rapid root establishment in the subsoil during the intensive biomass production period might be beneficial (Neukirchen et al. 1999) in case of early nutrient deficiency (Peng et al. 2012).

Share of biopore roots shown in this study was relatively lower compared with previous findings. Perkons et al. (2014) found approximately 20 % of root share inside biopores with winter barley. Nakamoto (1997) reported with 33 % of maize roots inside artificially created biopores. It indicates that root growing pattern via biopores highly depends on the pore properties (Hirth et al. 2005), crop species, and cropping duration (Jakobsen and Dexter 1988), as well as site conditions (Hatano et al. 1988; Kautz et al. 2013b). Considering our data on soil mineral N dynamics in the subsoil, the effects of N concentration on root growth are unlikely to assume. Also, the pore volume and bulk density did not indicate any effects of the measured soil physical property on root growth. Nevertheless, it shall not be completely ignored that other soil physical (e.g., soil moisture) and chemical (e.g., presence of nutrient patches) properties might have also affected the overall root growth at the study site (Davidson 1969). It has been shown that artificially created soil nutrient heterogeneity can lead to maximum sixfold of differences in rooting density of various shrub and grass species (Caldwell et al. 1996). Also, spatially and temporally applied fertilization and irrigation significantly affected root-length density and root biomass production of forest trees in which the

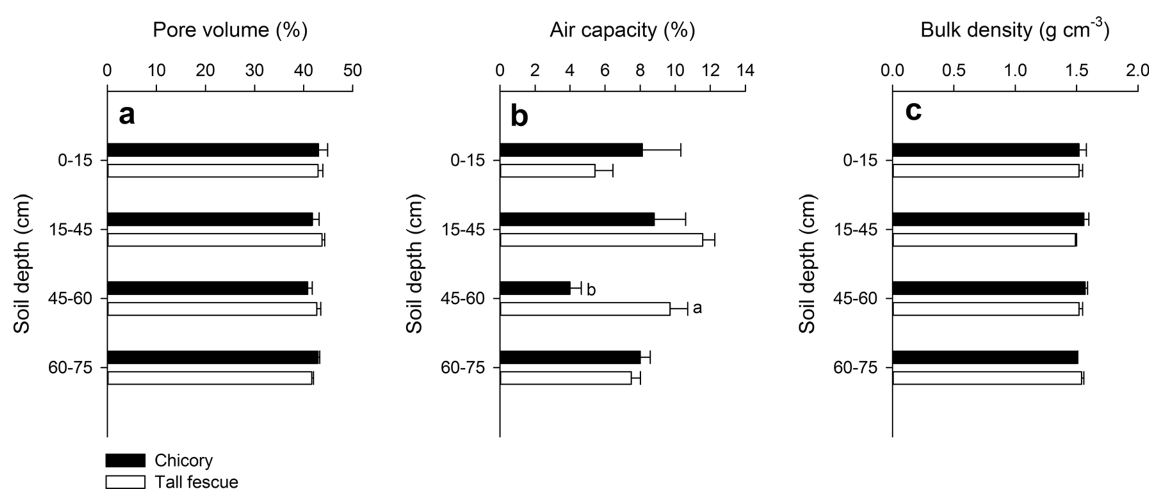

Fig. 3 Soil pore volume (**a**; %), air capacity (**b**; %), and bulk density (**c**; g cm^{-3}) after cultivation of chicory and tall fescue from 2010 to 2011 measured at from 0 to 75 cm of soil depth in 2012. *Different letters* indicate significant differences between two treatments (chicory and tall fescue) within the soil depth. Data were transformed for the analysis but mean values (± SE) are shown

Table 5 Univariate analysis on root/shoot growth parameters and soil N_{min} concentration as affected by crop sequence (CROP: chicory-wheat and tall fesuce-wheat) and crop growth stage (STAGE: tillering, booting, anthesis, and milk) of wheat in 2012

Variable	CROP	STAGE	CROP × STAGE
Root length inside biopores	7.821 *(≤0.006)*	4.185 *(≤0.006)*	1.162 (≤0.325)
Root length outside biopores	0.469 (≤0.494)	67.945 *(≤0.000)*	3.000 *(≤0.032)*
Root length inside/outside biopores	2.444 (≤0.119)	80.110 *(≤0.033)*	2.884 (≤0.717)
SPAD value	0.308 (≤0.585)	14.460 *(≤0.000)*	0.411 (≤0.747)
Leaf area index	0.444 (≤0.513)	26.627 *(≤0.000)*	0.981 (≤0.421)
Plant height	0.407 (≤0.531)	319.011 *(≤0.000)*	0.359 (≤0.783)
Shoot biomass	4.031 (≤0.058)	190.826 *(≤0.000)*	0.176 (≤0.911)
Shoot N uptake	0.846 (≤0.368)	21.584 *(≤0.000)*	0.540 (≤0.660)
Shoot P uptake	10.967 *(≤0.003)*	189.743 *(≤0.000)*	0.072 (≤0.974)
Shoot K uptake	0.001 (≤0.975)	18.879 *(≤0.000)*	0.535 (≤0.664)
Soil N_{min} concentration[a]	2.510 (≤0.188)	12.373 *(≤0.019)*	0.321 (≤0.746)

F values are shown with their probability levels in parentheses. Italicized *p* values indicate significant effects

[a] Measured at tillering, anthesis, and milk

effects of former were greater (Coleman 2007). In our study, higher amount of N, P, and K was accumulated as crop residues on topsoil after growing chicory than tall fescue, which might have also affected the overall nutrient demand and vertical root deployment strategy of wheat.

The enhanced P uptake of chicory-wheat sequence compared with tall fescue-wheat sequence might be an indirect evidence of enhanced mobilization potential with the increased rooting density in the subsoil (Kautz et al. 2013a) considering that the proportion of total P under Ap horizon accounted for 68 % at the study site (Barej et al. 2014). It might be also due to the exploitation of nutrient-rich drilosphere, "the 2-mm wide zone around the earthworm burrows" (Bouché 1975), with high contents of organic matter and microbial activity (Pierret et al. 1999; Kuzyakov et al. 2007). The former study (Barej et al. 2014) found that large sized biopores (>2 mm) and rhizosphere at the study site were enriched in resin and $NaHCO_3$-extractable inorganic P (P_i) and organic P (P_o) fractions at the soil depth of 30–75 cm. However, the accumulation of nutrients on upper parts of soil profile during the precrop phase should be also taken into account (Kautz et al. 2013a). In our study, higher amount of P was mulched by cultivation of chicory precrop in comparison with tall fescue. Thus, the increased P uptake by wheat can also be topsoil-driven as a function of mulching. Also, direct nutrient uptake from the pore wall might have had little relevance for the wheat in our field trial because relatively few roots grew in biopores compared to the bulk soil.

No significant effects of crop sequence shown on shoot growth parameters (SPAD value, LAI, and biomass yield) and final grain yield suggest that the expected biopore effects on overall crop performance might be more vividly shown under stress condition (Volkmar 1996; Gaiser et al. 2012). More importantly, understanding

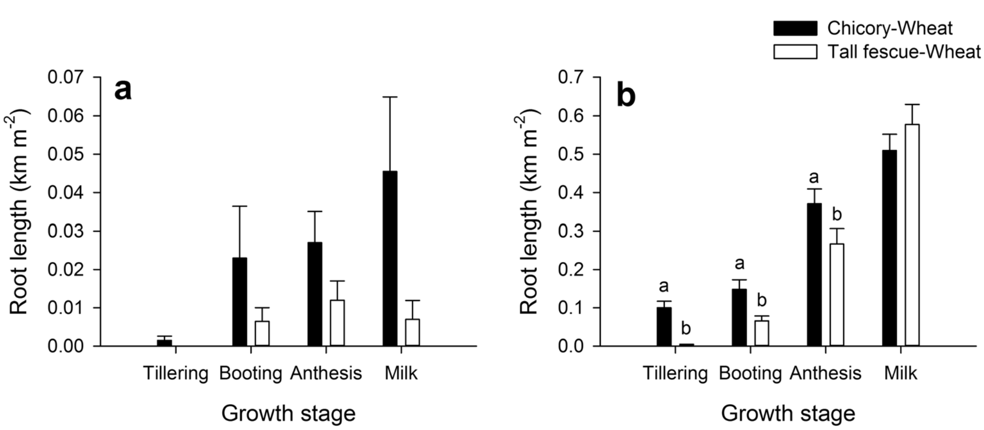

Fig. 4 Root length (km m^{-2}) inside (**a**) and outside (**b**) of biopores beneath 45 cm of soil depth as affected by crop sequence (chicory-wheat and tall fescue-wheat) and growth stage (tillering, booting, anthesis, and milk) in 2012. *Different letters* indicate significant differences between crop sequence treatments within growth stage (*t* test, $P \leq 0.05$). Root length inside biopore showed significant differences between crop sequence treatments across the growth stage. The data were transformed for the analysis but mean values (± SE) are shown. For results of univariate analysis, see Table 5

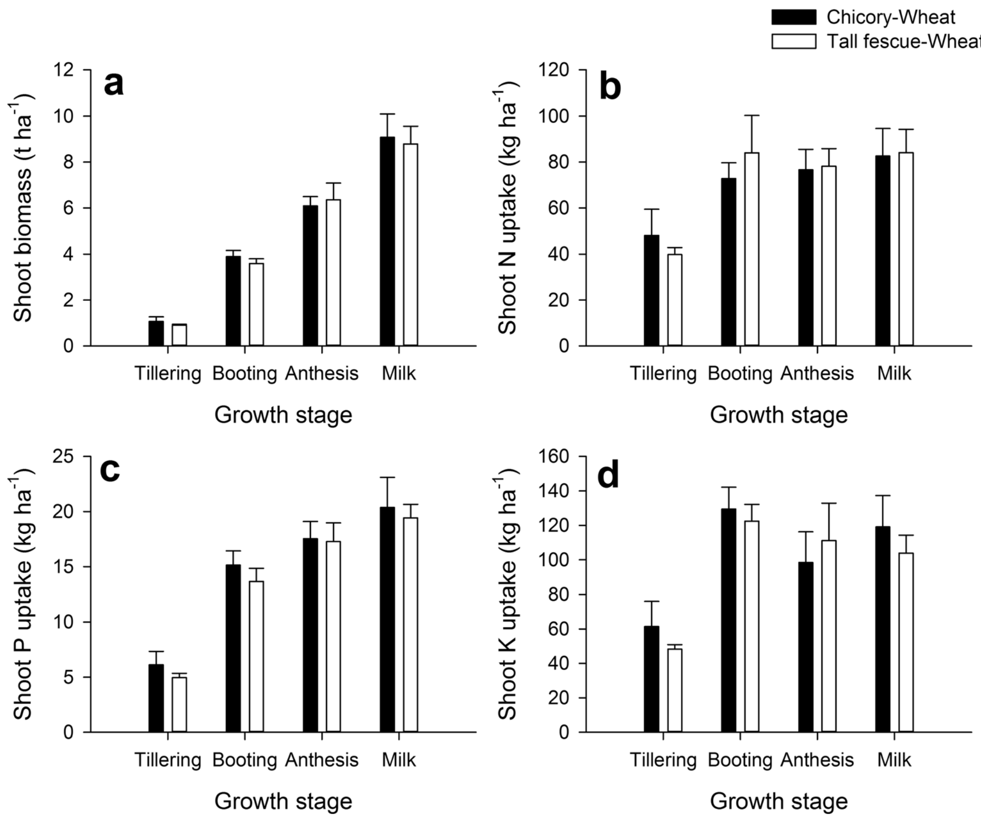

Fig. 5 Shoot biomass production (**a**; t ha^{-1}), N (**b**; kg ha^{-1}), P (**c**; kg ha^{-1}), and K uptake (**d**; kg ha^{-1}) of wheat as affected by crop sequence (chicory-wheat and tall fescue-wheat) and growth stage (tillering, booting, anthesis, and milk) in 2012. For results of univariate analysis, see Table 5

of biopore-root-shoot relationship might require simulation with mathematical modeling with myriad soil, plant, and environmental variables (Jakobsen and Dexter 1988; Gaiser et al. 2013).

Conclusions

We conclude that at the site under study biopores as pathways for rapid root growth into deeper soil layers allow roots to re-enter and explore the subsoil. Thus, cereals cultivated in rotation with taprooted crops can draw benefit from enhanced uptake of water and nutrients from deeper soil layers during early growth stages. Model simulations with various abiotic and biotic factors will be helpful to reveal the direct evidence of biopore-root-shoot relationship in the future.

Table 6 Yield (t ha^{-1}), N, P, and K uptake (kg ha^{-1}) of wheat grain and straw in 2012 after growing chicory (chicory-wheat) and tall fescue (tall fescue-wheat) for two consecutive years from 2010 to 2011

Yield parameter	Plant part	Crop sequence	
		Chicory-wheat	Tall fescue-wheat
Yield (t ha^{-1})	Grain	4.07±0.24	3.89±0.13
	Straw	3.21±0.26	3.25±0.12
N uptake (kg ha^{-1})	Grain	64.12±4.50	61.93±2.31
	Straw	11.18±1.48	9.40±0.93
P uptake (kg ha^{-1})	Grain	13.52±0.80	13.06±0.54
	Straw	2.99±0.24	2.39±0.14
K uptake (kg ha^{-1})	Grain	19.76±0.94	19.11±0.61
	Straw	24.22±2.64	20.64±0.86

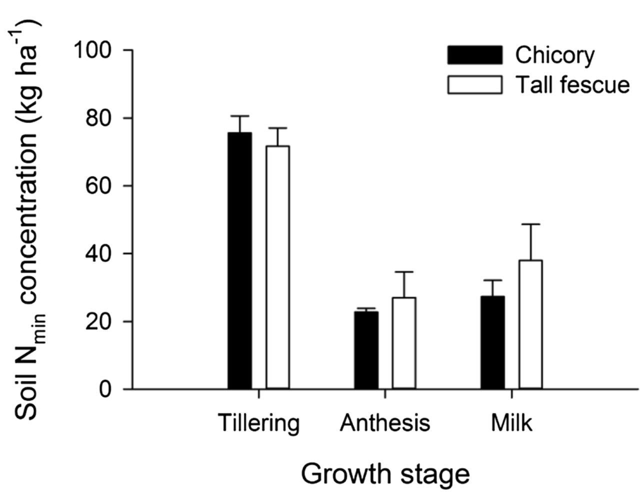

Fig. 6 Soil N_{min} concentration (kg ha^{-1}) at soil depth of 45–105 cm affected by crop sequence (chicory-wheat and tall fescue-wheat) and growth stage (tillering, anthesis, and milk) in 2012. The data were transformed for the analysis but mean values (± SE) are shown. For results of univariate analysis, see Table 5

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Conflict of interest The authors declare that they have no conflict of interest.

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