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REGULAR ARTICLE

Quantification of soil biopore density after perennial fodder cropping

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Abstract
Aims Plant root traits affect soil biopore (BP) formation. Aims of this study were to measure the effects of fodder crop species with contrasting root traits and duration of cropping on BP density (BPD), and also to address the consistency of these effects over different years focusing on the effects of root decay.
Methods Soil BPD was quantified after growing three perennial fodder crop species with contrasting root systems, namely, lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.) and tall fescue (*Festuca arundinacea* Schreb.) for 1, 2, and 3 years with 2 years fallow in two repeated field trials from 2007 to 2014.
Results Total BPD after taprooted fodder crops (421± 14 m^{-1}) was significantly higher compared with fibrous-rooted crops (337±12 m^{-1}). Cropping duration did not affect soil BPD. On average, density of medium-sized BP (BP_{med}; 2–5 mm) increased 14 % after 2 years of fallow, whereas BPD decreased by 5 % for coarse-sized BP (BP_{cor}; >5 mm) after the fallow.
Conclusions Taprooted fodder crops enhanced BP formation into subsoil. Accurate assessment of biopores (BPs) and their persistence must take account of the temporal dynamics, including effects of root decay.

Keywords Subsoil · Root system · Earthworm · Fodder crop · Pore dynamics

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Introduction

Subsoil is a hidden but important part of the soil profile that contains significant amount of soil nutrients (Rumpel and Kögel-Knabner 2010; Vancampenhout et al. 2012; Kautz et al. 2013a) and water (Kirkegaard et al. 2007; Lynch and Wojciechowski 2015) that can potentially be utilized for crop growth. Crop plants tend to increase root growth in deeper soil layers particularly when facing limitations of growth factors in the upper soil layers (Volkmar 1996; Gaiser et al. 2012). Subsoil resources are of particular importance for farming practices aiming at minimum use of external inputs such as Organic Agriculture (Köpke 1995; Gentile et al. 2005; Clark et al. 2007; Bell et al. 2012; Zotarelli et al. 2012). However, long-term use of heavy machinery (Batey and McKenzie 2006; Batey 2009; Hartmann et al. 2012) and inappropriate tillage practices (Taboada et al. 1998; Altikat and Celik 2011) often result in subsoil compaction. It has been extensively demonstrated that unfavorable soil conditions can impair root elongation (Jakobsen and Dexter 1988; Stirzaker et al. 1996). Suppressed root growth in the subsoil adversely affect acquisition of nutrient (Whiteley and Dexter 1982), thus resulting in poor crop performance (Atwell 1990).

Biopores (BPs) are round-shaped void channels in the soil created by plant roots and soil faunal activity (Jakobsen and Dexter 1988; Kautz et al. 2014; Perkons et al. 2014). The term often describes pores larger than 2 mm in diameter, but fine pores smaller than 0.2 mm in diameter have been considered as BPs by some authors (e.g. Volkmar 1996). BPs can be utilized for preferential root growth (Ehlers et al. 1983; Whiteley and Dexter 1983; Hatano et al. 1988; Stewart et al. 1999; Wuest 2001; Arora et al. 2011) that might enhance subsoil resource acquisition potential of crop plants (see review by Kautz 2014). Recent studies reported on increased rooting density (Perkons et al. 2014) of winter barley and improved water uptake by spring wheat as a function of increased number of BPs (Gaiser et al. 2012). Additionally, BPs are known to facilitate soil microbial activity (Vinther et al. 1999; Uksa et al. 2014) and soil air movement (Roseberg and McCoy 1990; Angers and Caron 1998). On the other hand, it has been also demonstrated (e.g. Passioura and Stirzaker 1993) that crop roots utilizing BPs did not lead to an enhanced plant growth (see review by Cresswell and Kirkegaard 1995).

Effects of fodder crops in cropping systems, especially the roles of N-fixing legumes and their ability to supply additional N to the subsequent crops have been extensively studied (see reviews by van Kessel and Hartley 2000; Crews and Peoples 2004). Grain legumes such as faba bean and soy bean were reported with their contribution of 155 and 280 kg ha^{-1} of N, respectively, to the soil after grain harvest (Rochester et al. 2001). Net soil N balance of 1-, 2- and 3-year stands of lucerne ranged from 83 to 148 kg ha^{-1} (Kelner et al. 1997). Grain yield of subsequently grown maize and wheat increased 24 % after pigeon pea (Rao and Mathuva 2000) and over 100 % after mung bean (Bakht et al. 2009), respectively. Apart from N effects, increased soil organic C with residue retention (Al-Kaisi et al. 2005; Gentile et al. 2005; Sainju and Lenssen 2011; Zotarelli et al. 2012), suppressed weed growth (Mertens et al. 2002; Hiltbrunner et al. 2007; Chikoye et al. 2008) and more efficient disease and pest management (Mueller et al. 2005; Govaerts et al. 2006) were also reported as important effects of crop sequence with fodder crops.

Previous studies have shown that fodder crops with deep taproots can enhance biopore (BP) formation (e.g. McCallum et al. 2004; Kautz et al. 2014; Perkons et al. 2014) and increase BP density (BPD: number of BP per unit area) in agricultural subsoil. The BP formation process is influenced by factors such as root diameter, root-length density and the abundance of anecic earthworms (Materechera et al. 1992; Kautz et al. 2014). However, information on the mechanistic relationship between those traits is still lacking due to the complexity of interactions among the causal factors (see reviews by Cresswell and Kirkegaard 1995; Kautz 2014).

Also the extent to which BPD measurements are affected by the decomposition of root material inside the BP overtime (Dexter 1991; Jones et al. 2004; McCallum et al. 2004; Pagenkemper et al. 2014) has not been well documented. This study aims to (i) measure the effects of fodder crop species and duration of cropping on BPD and (ii) investigate the effect of root decay on the quantification of BPD over time. We hypothesized that (a) fodder crop species differed in their impacts on BPD; (b) that the effects of species and duration of cropping would be consistent overtime; and (c) BPD would increase after a period of 2 years has elapsed since fodder crops are terminated as a result of root decay.

Materials and methods

Experimental site

The investigations were carried out at the Campus Klein-Altendorf research station located in Rheinbach, Germany (50°37′9″ N, 6°59′29″E). The soil was classified as Haplic Luvisol (Hypereutric, Siltic) developed from loess (IUSS Working Group WRB 2006). The annual mean temperature and precipitation recorded from 1956 to 2010 were 9.4 °C and 603.4 mm, respectively. Annual means of air/soil temperature (°C) and precipitation (mm) from 2007 to 2014 are shown in Table 1.

Profile trenches were opened prior to the investigation and their physical and chemical properties were analyzed along the soil profile (0-116+ cm soil depth). Six distinct horizons defined were Ap (0–31 cm), A1/Bt (31–42 cm), Bt1 (42–63 cm), Bt2 (63–86 cm), Bwt (86–116 cm) and leCw (116+ cm). Investigation on root, anecic earthworm and BPD was carried out at 45 cm of soil depth where B horizon commenced. Detailed information on the soil properties was presented by Vetterlein et al. (2013).

Table 1 Annual air/soil temperature and precipitation in 2007–2013 at the study site

Measurement	2007	2008	2009	2010	2011	2012	2013	2014
Mean air temperature (°C)	10.7	10.0	10.1	8.8	10.7	9.9	9.4	11.2
Mean soil temperature (°C)[a]	12.3	12.5	12.3	11.9	12.8	12.2	12.0	13.7
Annual precipitation (Σ mm)	750.6	601.3	603.1	607.4	544.6	602.1	607.1	717.5

[a] Measured at 20 cm of soil depth

Treatments

As shown in Table 2, the experiment consisted of two identical field trials (factor: TRIAL), viz., Trial A (TA: 2007–2012) and Trial B (TB: 2009–2014). In each trials, three different fodder crop species, viz., lucerne (*Medicago sativa* L. 'Planet') with taproot system and nodules, chicory (*Cichorium intybus* L. 'Puna') with taproot system, and tall fescue (*Festuca arundinacea* Schreb. 'Hykor') with fibrous root system (factor: CROP) were grown for 1, 2 and 3 years (factor: DUR) during the first 3 years of experiment. The sowing density was 25, 5 and 30 kg ha^{-1} for lucerne, chicory and tall fescue, respectively. Fodder crops were cut up to four times in each vegetation period, and the shoot materials were left on site. No tillage operation was carried out during fodder cropping. Initial BPD investigation was done in 2010 and 2012 in TA and TB, respectively. Final investigation was carried out in 2012 (TA) and 2014 (TB) after 2 years of fallow practice (factor: FALLOW). Field experiment design was strip-plot with 36 plots in four blocks. The single plot size was 6m × 10 m.

Sampling

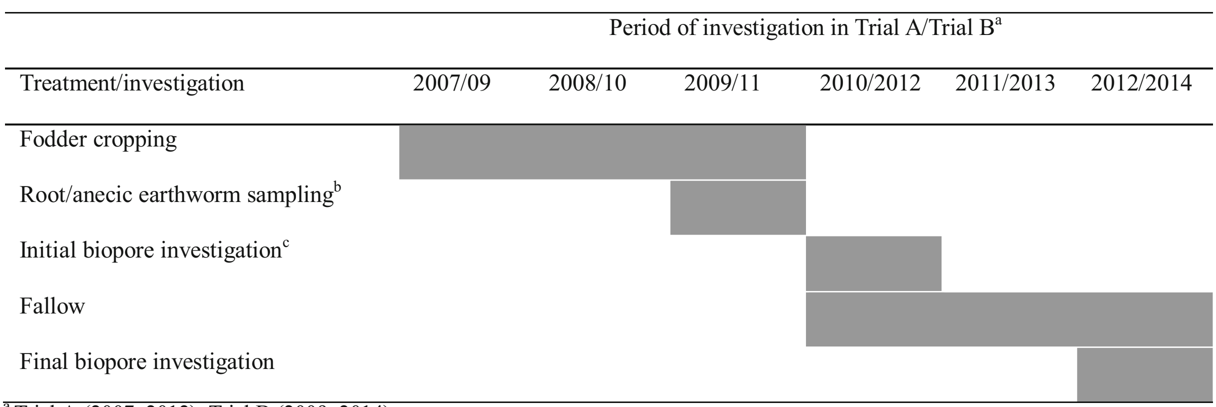
Root sampling

In 2011, four replicated soil monoliths of 2500 cm^{-3} (25 cm × 10 cm × 10 cm) were collected in TB at 45–55 cm soil depth. Sampling spots inside plots were restricted to where the distance to each border was at least 3 m apart. The collected samples were carefully washed with tap water, and debris was removed. The sorted roots were photo-scanned. Images were analyzed with the software 'WinRHIZO Pro' (Version 2009c, 32 Bit) to determine root diameter (mm) and root-length density (RLD: cm cm^{-3}).

Anecic earthworm extraction

The mustard extraction method (Gunn 1992) was adopted for determining the biomass (g m^{-2}) and abundance (individuals m^{-2}) of anecic earthworms, viz., the deep burrowing earthworm species, in TB in autumn 2011. *Lumbricus terrestris,* the only anecic earthworm species present at the experimental area, was

Table 2 Timeline of the experiment from 2007 to 2014



[a] Trial A (2007–2012); Trial B (2009–2014)

[b] Root/anecic earthworm sampling was done only in Trial B

[c] Biopore investigation was done from March to April at each investigation in both trials

investigated. Mustard solution was prepared with 10 l of tap water and 125 g mustard (type: Düsseldorfer Löwensenf) and was poured onto the soil surface twice with an interval of 5–10 min via a metal frame (0.5 m× 0.5 m) that was inserted in the upper soil layer. Earthworms appearing within 30 minutes after the application of the mustard solution were collected into water-filled boxes and stored for later identification, counting and weighing.

BPD investigation

Areas larger than 0.25 m^2 were excavated to a soil depth of 45 cm inside the plots. Then the surface area was carefully flattened and cleaned with vacuum cleaner to reveal the soil BPs. A transparent plastic sheet of 50 cm ×50 cm size was fitted on the prepared subsoil surface, and all BPs visible through the plastic sheet were marked. Coarse-sized BP (BP_{cor}: >5 mm) and medium-sized BP (BP_{med}: 2–5 mm) were distinguished with felt pens of different colors. After each initial investigation, a fabric cover was laid on the soil surface in 45 cm soil depth, and the sampling area was refilled with soil. During the bare fallow phase, any plant growth was suppressed by mechanical hand weeding for 2 years until further investigation.

Statistical analysis

R (Version 3.0.2, 64-bit) was used for statistical analysis. Prior to any specific analysis, the data were tested with Shapiro-Wilk normality test ($P\leq 0.05$). Based on that, root traits, anecic earthworm abundance/biomass data were log-transformed. Principal component analysis (PCA) was adopted as a multivariate approach to briefly measure the effects of crop species and cropping duration by grouping the treatment subjects based on four variables considered relevant for BP formation (root diameter, RLD, abundance and biomass of anecic earthworm). The number of principal components was determined with eigenvalue (Kaiser 1960). BPD data acquired from TA were partially used by Kautz et al. (2014) in which mean BPD was calculated and used for comparisons between the treatments. In this paper, data from both trials were collected, and a linear mixed-effects model (Pinheiro and Bates 2000) was used for univariate analysis. Where necessary, post-hoc tests (Tukey's HSD, $P\leq 0.05$) were performed.

Results

Root traits and abundance/biomass of anecic earthworms

PCA with root diameter and RLD of the fodder crop species and abundance as well as biomass of anecic earthworm revealed two main components (PC1 and PC2). PC1 and PC2 explained 44.1 and 35.4 % of total variance, respectively (Table 3). PC1 captured the earthworm-related parameters, while PC2 was related to root diameter and RLD (Fig. 1). According to the PC score, clear groupings of the treatment objects with three fodder crop species and three cropping durations were observed. Objects from each fodder crop species were grouped based on the root-related parameters along PC2. Chicory objects were located in the positive part of PC2 and tall fescue objects in the negative part of PC2. Objects from lucerne treatments remained at the intermediate position. Along PC1, the longer duration treatments tended to group on the positive side of the biplot, which was based on the abundance of anecic earthworms. The tendency for such grouping was more strongly shown for lucerne treatments followed by chicory and tall fescue.

Based on the univariate analysis, root diameter and RLD of the three fodder crops measured at 45–55 cm soil depth differed (Table 4). However, the differences were associated with a significant interaction with cropping duration. Thus, post-hoc tests (Tukey's HSD, $P\leq 0.05$) between the fodder crop species at each duration were carried out. When cultivated for 1 year and 2 years, root diameter of the three fodder crop species

Table 3 Component matrix obtained from principal component analysis (PCA) with root-length density (RLD) and root diameter of fodder crop species in 2011 and abundance and biomass of *Lumbricus terrestris* in 2012

Traits	PC1 (44.1 %)	PC2 (35.4 %)
RLD	0.449	−0.724
Root diameter	−0.188	0.853
Abundance of *Lumbricus terrestris*	0.862	0.345
Biomass of *Lumbricus terrestris*	0.886	0.212

For distribution of objects based on PC1 and PC2, see Fig. 1

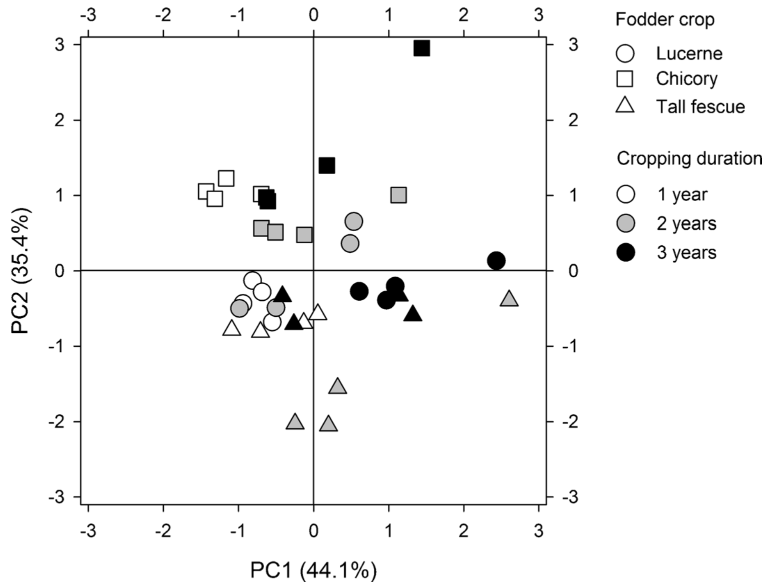


Fig. 1 BP formation: Principal component analysis (PCA) of four underlying factors (root-length density, root diameter, abundance and biomass of *Lumbricus terrestris*)

significantly differed whereby chicory produced the largest roots followed by lucerne and tall fescue (Fig. 2a). In the 3rd year of cropping, these differences in root diameter between lucerne and tall fescue were no longer observed. Nevertheless, chicory maintained the widest roots in the 3rd year. RLD of the three fodder crops at each cropping duration also significantly differed (Fig. 2b). At the 1st and 3rd year of cropping, lucerne and tall fescue gave higher RLD compared with chicory. For the 2 years treatments, tall fescue had high RLD suggesting significant differences between fibrous and taprooted crops.

Biomass and abundance of anecic earthworm measured prior to BP investigation in 2012 showed significant effects of DUR but not of CROP (Table 5). Differences in crop species did not affect the biomass of anecic earthworm (Fig. 3a). In descending order, earthworm biomass was the highest after 3 years (28.7 g m^{-2}) followed by 2 years (26.1 g m^{-2}) and 1 year (9.4 g m^{-2}) of cropping duration (Fig. 3b). Earthworm abundance was highest after 3 years of cropping (51.0 m^{-2}) compared with 2 years (22.3 m^{-2}) and 1 year treatments (8.0 m^{-2}; Fig. 3d). In contrast to DUR, the effect of CROP did not have influence across the crop species treatments (Fig. 3c).

Table 4 Univariate analysis on root diameter (mm) and root-length density (RLD, cm cm^{-3}) of three fodder crop species (CROP: lucerne, chicory and tall fescue) during three periods of cropping duration (DUR: 1, 2 and 3 years) in 2009-2011

Source	df	Root diameter	RLD
CROP	2	79.983 (**≤0.000**)	73.788 (**≤0.000**)
DUR	2	0.492 (≤0.634)	25.800 (**≤0.001**)
CROP × DUR	4	3.933 (**≤0.029**)	4.728 (**≤0.016**)

Data were log-transformed. F-values are shown with their probability levels in parentheses. Bold-face p-values indicate significant effects

Soil biopore density

Univariate analysis (Table 6) revealed that BPD_{tot}, viz., all BP >2 mm diameter, in the subsoil was significantly affected by CROP. Mean comparisons (Tukey's HSD, $P \leq 0.05$) resulted in higher BPD_{tot} of taproot system treatments (lucerne and chicory) in comparison to the fibrous root system (tall fescue; Fig. 4a). BPD_{tot} after lucerne, chicory and tall fescue cultivation were 437, 406 and 336 m^{-2}, respectively. The number of medium-sized BP (BPD_{med}, 2–5 mm) and coarse-sized BP (BPD_{cor}, >5 mm) was also significantly affected by CROP. Lucerne (256 m^{-2}) and chicory treatments (176 m^{-2}) resulted in higher BPD_{med} compared with tall fescue (149 m^{-2}) across DUR. For BPD_{cor}, significant differences in BPD remained for lucerne and tall fescue, and mean values of BPD_{cor} after lucerne, chicory and tall fescue treatments were 181, 176 and 149 m^{-2}, respectively. Cropping duration

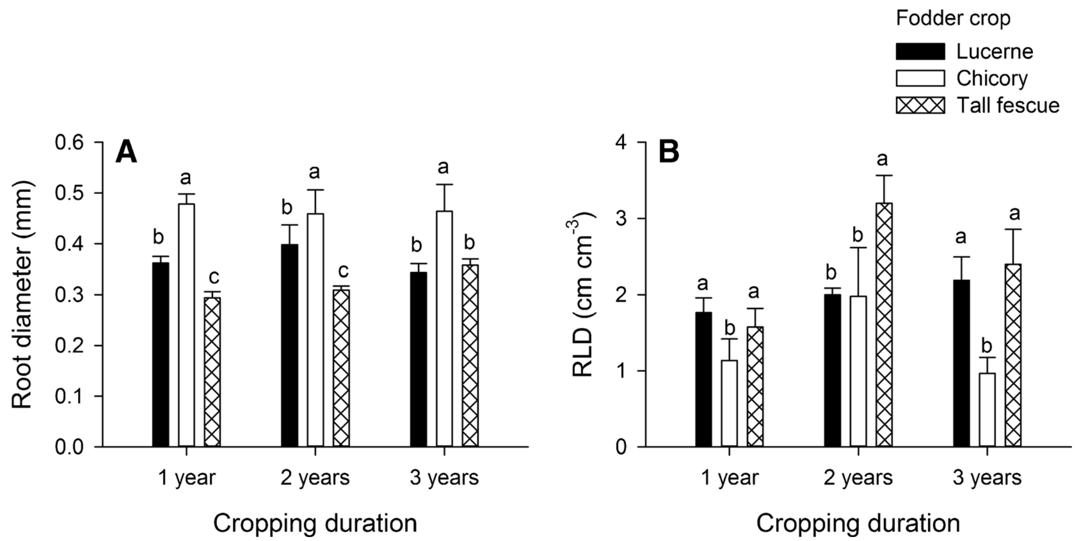


Fig. 2 Root diameter (**A**; mm) and root-length density (**B**; RLD, cm cm^{-3}) of lucerne, chicory and tall fescue in 45–55 cm soil depth at each duration of fodder cropping (2009–2011). *Roman letters* indicate significant differences between fodder crops within cropping duration (Tukey's HSD, $P \leq 0.05$). The data were log-transformed for the analysis but mean values (± one SE) are shown. For results of univariate analysis, see Table 4

as a factor did not have influence on BPD of all three classes (Table 6; Fig. 4b).

Quantification of BPD was significantly affected by TRIAL. BPD_{med} in TA (246 m^{-2}) was higher than in TB (205 m^{-2}; Fig. 6a). BPD_{cor} showed significant interaction between TRIAL and FALLOW (Fig. 5b). Comparisons of BPD_{cor} between the trials before and after fallow practice revealed significant differences in which BPD_{cor} in TB was higher compared with TA. However this difference was not observed before fallow. BPD_{tot} did not show any effect of TRIAL (Fig. 5a).

Effects of FALLOW were different for the two classes of BP (Table 6). The 2 years of fallow practice increased BPD_{med} across all trials (Fig. 6b). BPD_{cor} resulted in inconsistent pattern of variation between the two trials. BPD_{cor} in TA decreased when fallowed, whereas it did not change in TB (Fig. 5b). As a result, BPD_{tot} was not affected by the fallow practice in both trials (Fig. 5a).

Discussion

Root traits

PCA of root traits (root diameter and RLD) revealed distinguished root growth patterns (Bodner et al. 2013) of the different fodder crop species (Fig. 1). Root diameters of lucerne and chicory were larger than root diameters of tall fescue (Fig. 2a) indicating differences between taproot and fibrous root systems (Materechera et al. 1992). This root morphological difference is reflected by the ability of taproot systems to create more soil BPs with diameters at least larger than 2 mm (Athmann et al. 2013; Kautz et al. 2014; Perkons et al.

Table 5 Univariate analysis on biomass (g m^{-2}) and abundance (individuals m^{-2}) of *Lumbricus terrestris* in 2012 after growing three fodder crop species (CROP: lucerne, chicory and tall fescue) for three periods of cropping duration (DUR: 1, 2, and 3 years) from 2009 to 2011

Source	df	Biomass of *Lumbricus terrestris*	Abundance of *Lumbricus terrestris*
CROP	2	0.011 (≤0.989)	0.883 (≤0.428)
DUR	2	3.881 **(≤0.035)**	19.171 **(≤0.000)**
CROP × DUR	4	0.988 (≤0.433)	1.564 (≤0.220)

Data were log-transformed. F-values are shown with their probability levels in parentheses. Bold-face p-values indicate significant effects

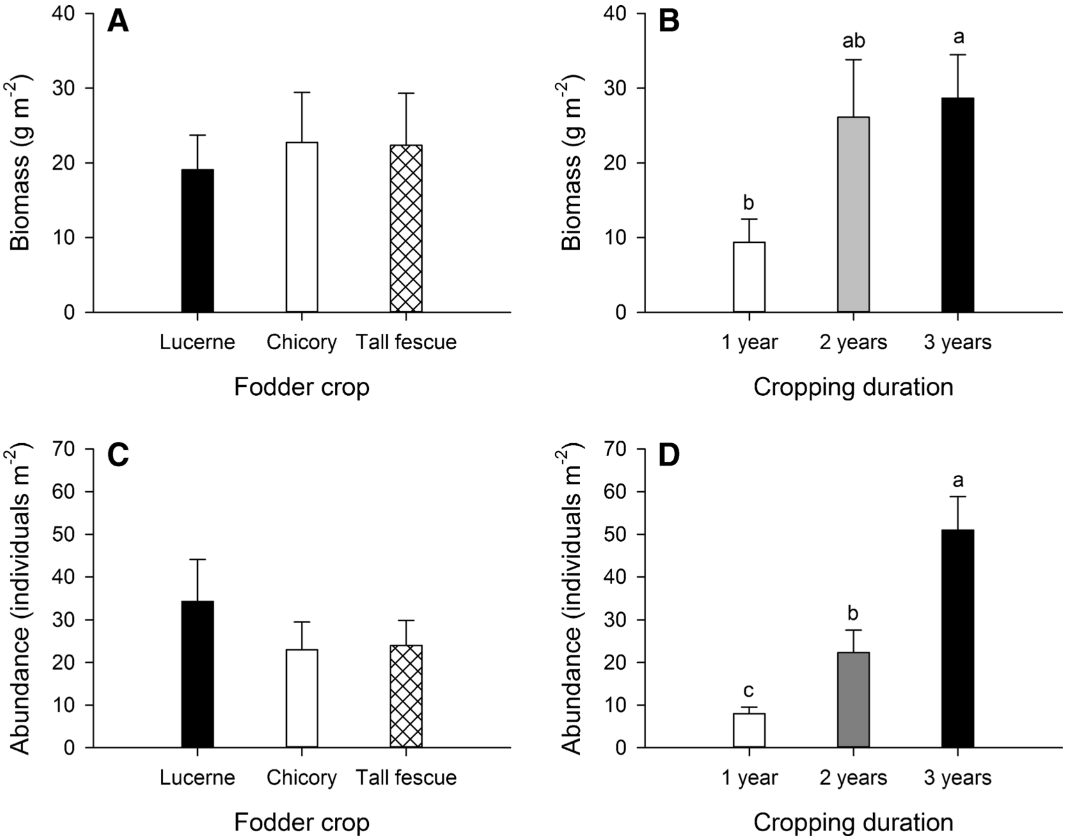


Fig. 3 Biomass (**A** and **B**; g m^{-2}) and abundance (**C** and **D**; individual m^{-2}) of *Lumbricus terrestris* affected by fodder crops (**A** and **C**) and cropping duration (**B** and **D**). *Small letters* indicate significant differences between treatments (Tukey's HSD, $P \leq 0.05$). Differences are not significant without indication. The data were log-transformed for the analysis but mean values (± one SE) are shown. For results of univariate analysis, see Table 5

Table 6 Univariate analysis on biopore density (BPD; number m^{-2}) larger than 2 mm (BPD_{tot}), 5 mm (BPD_{cor}) and 2–5 mm (BPD_{med}) after growing three fodder crop species (CROP: lucerne, chicory and tall fescue) for three periods of cropping duration (DUR: 1, 2, and 3 years) in two different trials (TRIAL: TA and TB) with fallow practice (FALLOW: before and after) in 2009–2014

Source	df	BPD_{tot}	BPD_{cor}	BPD_{med}
CROP	2	14.592 **(≤0.000)**	4.324 (≤0.055)	10.073 **(≤0.000)**
DUR	2	0.419 (≤0.670)	0.341 (≤0.720)	0.267 (≤0.772)
TRIAL	1	0.815 (≤0.369)	10.824 **(≤0.002)**	11.087 **(≤0.001)**
FALLOW	1	1.890 (≤0.172)	1.268 (≤0.264)	6.048 **(≤0.016)**
CROP × DUR	4	1.031 (≤0.396)	1.450 (≤0.291)	0.344 (≤0.847)
CROP × TRIAL	2	1.623 (≤0.203)	2.087 (≤0.131)	0.519 (≤0.597)
DUR × TRIAL	2	1.007 (≤0.369)	1.611 (≤0.206)	0.692 (≤0.503)
CROP × FALLOW	2	1.538 (≤0.220)	0.563 (≤0.572)	1.611 (≤0.205)
DUR × FALLOW	2	0.208 (≤0.813)	0.445 (≤0.643)	0.021 (≤0.980)
TRIAL × FALLOW	1	0.285 (≤0.595)	8.193 **(≤0.005)**	1.604 (≤0.208)

F-values are shown with their probability levels in parentheses. Bold-face p-values indicate significant effects. 3-and 4-way interactions were not significant

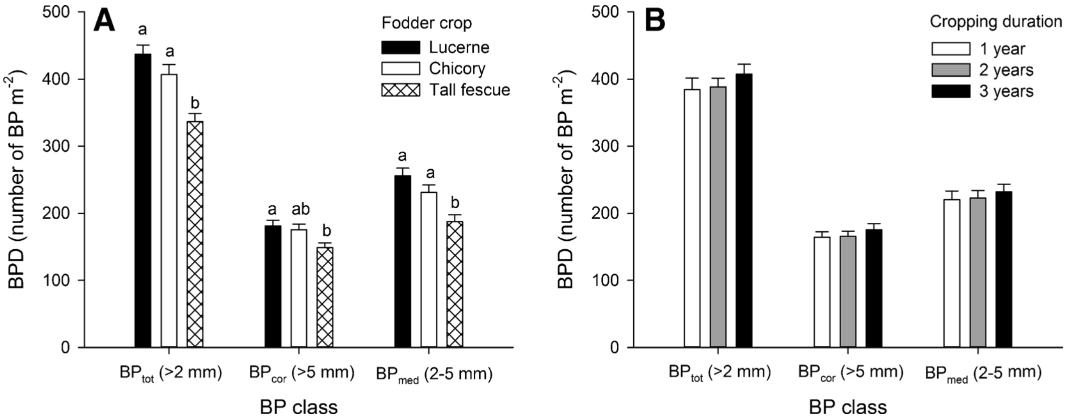


Fig. 4 Biopore density (BPD; mean±one SE) of all size classes (BP_{tot}: >2 mm), coarse-sized (BP_{cor}: >5 mm) and medium-sized (BP_{med}: 2–5 mm) affected by fodder crops (**A**: lucerne, chicory and tall fescue) and cropping duration (**B**: 1, 2 and 3 years). *Small letters* indicate significant differences between the treatments within BP class (Tukey's HSD, $P \leq 0.05$). Differences are not significant without indication. For results of univariate analysis, see Table 6

2014). In contrast to the different root diameters of the three fodder crops, the fibrous root system of tall fescue showed higher RLD than taproot systems of lucerne and chicory (Fig. 2b). These results are in accordance with the earlier findings on extensive rooting habit of tall fescue (White et al. 1993; Carrow 1996).

Abundance and biomass of anecic earthworm

The higher abundance/biomass of anecic earthworms as a function of cropping duration (Fig. 3b and d) is considered as a function of soil rest (Binet et al. 1997) and high amounts of feed for earthworms made available by shoot residues (Riley et al. 2008) left on-site as mulch. Non-significant effects of different fodder crop species on soil anecic earthworm abundance and biomass are in accordance with Kautz et al. (2014). It can be speculated that *Lumbricus terrestris* did not prefer any of the shoot and material from different species. These results can be related to the findings of Bonkowski et al. (2000) who reported feed preference of anecic earthworms to different fungal species but not to relatively fresh plant residues. As a result, no correlation between anecic earthworm biomass/abundance and BPD was observed (data not shown). Thus, the initial BP formation is considered as a function of crop root penetration (Kautz et al. 2014; Perkons et al. 2014), which is followed by the utilization of these root-made BPs by soil anecic earthworms (Pagenkemper et al. 2014).

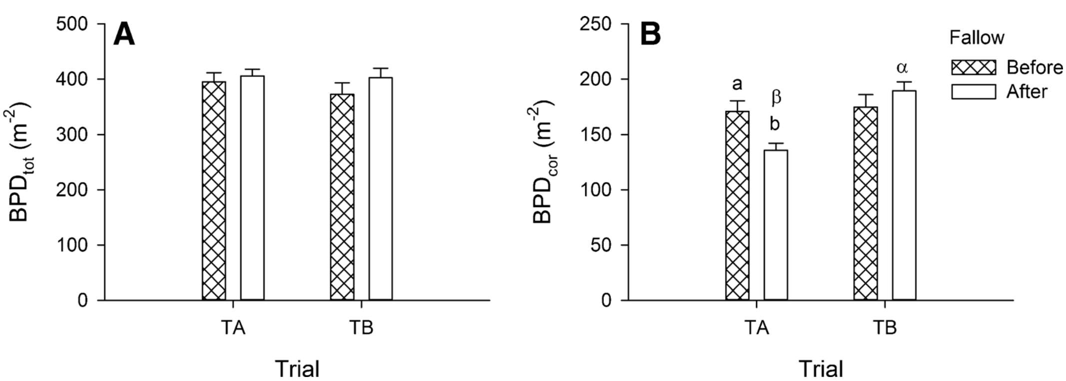


Fig. 5 Density (number m^{-2}; mean±one SE) of total (**A**; BPD_{tot}) and coarse-sized biopore (**B**; BPD_{cor}) between two trials (TA and TB) and fallow practice (before and after). *Roman and Greek letters* indicate significant differences between the fallow practice and trials, respectively (Pair-wise t-test, $P \leq 0.05$). Differences are not significant without indication. For results of univariate analysis, see Table 6

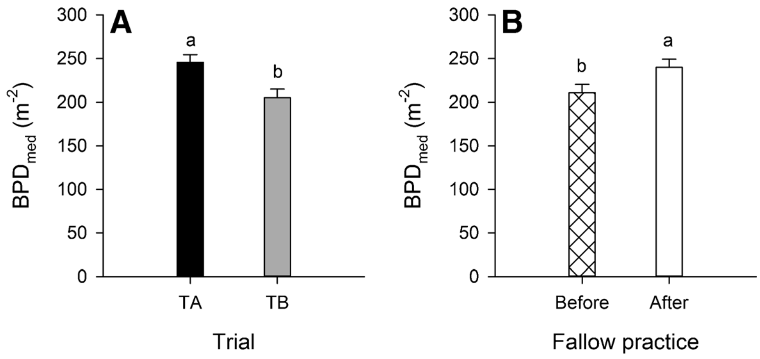


Fig. 6 Density (number m^{-2}; mean±one SE) of medium-sized biopore (BPD_{med}) affected by trial (**A**: TA and TB) and fallow practice (**B**: before and after). Small letters indicate significant differences (Pair-wise t-test, $P \leq 0.05$). For results of univariate analysis, see Table 6

Cropping system and BPD

The experiments revealed clear effects of fodder crop species and their corresponding rooting patterns on BPD in the subsoil (Table 6). The significantly higher BPD after the taprooted lucerne and chicory compared with the fibrous-rooted tall fescue has been reported in recent studies at the same site (Athmann et al. 2013; Kautz et al. 2014; Perkons et al. 2014). As confirmed by the multivariate approach with PCA, the larger root diameter of the taprooted species may have enabled better penetration into the subsoil at the study site (Materechera et al. 1992). However, lucerne with smaller root diameter resulted in higher BPD_{cor} than chicory with larger root diameter (Fig. 2a) presumably as a function of the higher crop density. It can also be assumed that root diameter of coarse primary and lateral roots of lucerne might have been more homogenous in comparison to chicory, which could not be detected under this study. Significantly higher water uptake of spring wheat after perennial lucerne cultivation observed on the same sites by Gaiser et al. (2012) can be related to the high BP forming potential of lucerne roots as observed in the current experiment. Distribution pattern of different root size classes (McCallum et al. 2004) and root architectural traits (Fitter 1987; Nuttall et al. 2008) might have also resulted in the differences in BPD between the similar root systems; however, this requires further investigation to confirm.

Despite the tendency for a slight increase of BPD over cropping duration, the effects were not significant (Table 6). However, considering the stable pore system over decades in the subsoil (Hagedorn and Bundt 2002) and the observed increase in anecic earthworm abundance and biomass over cropping duration, further research with a longer period of time might be needed for the effects to be revealed.

Variation in BPD quantification

BPD_{med} between the two trials locations differed. Considering the different period of investigation on the trials (TA: 2007–2012 and TB: 2009–2014), the variation is likely to be temporal-driven with abiotic factors such as weather conditions at the study site (see Table 1). Smethurst et al. (2012) reported on the variation in soil pore water pressures in upper part of clay soils, especially during summer rainfall, which was related to frequent shrinking and swelling in soils due to seasonal cycles of soil water content.

Effects of fallow practice on BPD differed for the two BP classes. The effects of complete decay of roots on BPD_{med} (Fig. 6b) in TA and TB suggests underestimation of BPD when filled with fresh root residues (Ehlers 1975; Dexter 1991; Binet et al. 1997; Jones et al. 2004; Kautz et al. 2014; Perkons et al. 2014). Visibility through plastic film upon investigation, especially in natural environments is impaired with the fillings. The higher BPD_{cor} upon the initial investigation in TA compared to the later investigation is not in accordance with our hypothesis. It can be related to the temporal dynamics of pore-size distribution (Leij et al. 2002). PSD is affected by abiotic (e.g. wetting and drying of soil) and anecic earthworm activity (Pagenkemper et al. 2014), which might have caused the collapse of relatively unstable coarse BPs in TA resulting in smaller pore size after fallow. Additionally, since the lower BPD_{cor} determined in TA after fallow practice in the later investigation (Fig. 5b) is accompanied by a strong increase in BPD_{med} (Fig. 6b), it might be an effect of human error. BPs were counted by different observers in the different years (before and after fallow). Therefore, it is possible that despite careful training, individual calibration of investigators was slightly different leading to a shift from BPD_{cor} to BPD_{med} in the second counting.

Soil BPD and crop yield

Increased rooting density with high soil BPD in the subsoil (Perkons et al. 2014) might enhance soil nutrient acquisition potential. Utilization of nutrient-rich and of biologically active drilosphere (Devliegher and Verstraete 1997; Stewart et al. 1999; Brown et al. 2000; Kautz et al. 2013a; Uksa et al. 2014) can also be a potential mechanism affecting the crop yield. However, drawing up conclusions on the relationship between BPD and crop performance should be made with caution due to its multitude processes involved (see review by Kautz et al. 2013a), which in general would require mathematical modeling (Jakobsen and Dexter 1988; Gaiser et al. 2013). For instance, even with the significant positive relationship found between pore density and shoot ^{15}N-uptake (r^2=0.57) of wheat, Volkmar (1996) regarded the relationship inconclusive due to the lateral roots growing inside and outside of pores. It was also often claimed that the effects of pore dynamics might be revealed better under stress condition (e.g. Gaiser et al. 2012). In fact, under favorable weather condition, a 3-year study by McCallum et al. (2004) has demonstrated the influence of pore density on crop yield of canola only in one season. Also, root elongation pattern inside BPs (Athmann et al. 2013) and ability of laterals to re-enter the bulk soil (Kautz et al. 2013b) might be important to consider as they determine soil-root contacts and thus, accessibility to the subsoil resources (see reviews by Cresswell and Kirkegaard 1995; Kautz 2014).

Future research

The variation in BPD between trials, period of investigation and observers suggest the need of methodological improvement of BPD quantification in the future. Adaptation of image-based methodology might provide valuable information. Precise measurement of BP diameter, area and density (Wuest 2001) can be automatically processed with good quality images from the field, which will avoid human errors upon calibration of different BP classes. Geostatistical analysis approach (Diggle 1983) with the digitized BP data would enable the observer to measure the level of distribution of BP, viz., regular, random and clustered (van Noordwijk et al. 2000), which might be a result of the various soil floral and faunal activities.

The relationship between BP and root growth is often measured *ex situ* (e.g. Hatano et al. 1988) due to practical hardships to observe the dynamics of the effects *in situ* (Hutchings and John 2003; Valentine et al. 2012). Thus, further investigations that measure and quantify the effects of BP on root elongation in the subsoil under field conditions (e.g. Perkons et al. 2014) will be necessary (Materechera et al. 1992; Valentine et al. 2012). Moreover, investigation on microbial and chemical properties (Pierret et al. 1999; Vinther et al. 1999; Brown et al. 2000; Pankhurst et al. 2002; Hinsinger et al. 2009) of the drilosphere (Bouché 1975), and development of techniques to link those properties to crop performance and rooting pattern will be of prime importance.

Conclusions

Overall, our data indicate that the value of including taprooted fodder crops into crop rotations can improve soil quality by enhanced BP formation. The results indicate that the initial BPD_{med} quantified immediately after cultivation of fodder crops can change over time, probably due to the appearance of the previously blocked BPs following root decay. This implies that precise quantification of the effects of root growth on BPD should not be undertaken before complete decay of the roots. Temporal dynamics should be also considered as an important factor upon quantification as our data revealed variation in BPD and pore-size distribution at different period of investigation. Weather conditions might be strong factors determining BP formation. Adoption of image-based methodology might be helpful to reduce the human errors and also to measure more quantitative parameters (e.g. diameter and area of individual BPs).

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Compliance with ethical standards

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