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# Contribution of anecic earthworms to biopore formation during cultivation of perennial ley crops

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ABSTRACT

Large sized biopores (diameter >2 mm) in the subsoil can be created by tap roots, which leave voids after their decay, or by the burrowing activity of anecic earthworms which may benefit from the temporary lack in tillage in perennial cropping systems. However, the interactions between root growth and earthworm activity in the process of biopore formation during perennial ley cropping are not well understood. The aim of this field study was to quantify the development of the abundance of the anecic earthworm *Lumbricus terrestris* and the biopore density during the cultivation of lucerne (*Medicago sativa* L.), chicory (*Cichorium intybus* L.) and tall fescue (*Festuca arundinacea* Schreb.) grown for either one, two or three years. An increased abundance of *L. terrestris* was already recorded after two years of continuous ley when compared with one year cultivation. The ley crop species had only minor influence on the abundance of *L. terrestris*. Biopore densities of both diameter classes under study (2–5 mm and >5 mm) were not significantly affected by the duration of ley cropping. In contrast, biopore densities were influenced by ley crop species. More biopores of diameter class 2–5 mm were recorded after chicory than after fescue. Lucerne cropping resulted in intermediate biopore density. Additionally, in an incubation experiment under field conditions, we quantified whether *L. terrestris* preferentially created new biopores or colonized abandoned, previously existing ones. After three weeks of incubation, one third of the adult individuals incubated in the experimental area created new biopores at 0.4 m soil depth. A similar percentage of individuals colonized previously existing biopores, partially widening the lumen of smaller sized biopores. The remaining individuals remained in the topsoil. Sub-adult individuals rarely formed new pores. Half of the introduced sub-adults remained in the topsoil. We conclude that in crop rotations new biopores can be generated during perennial ley cropping by taproot systems of ley crops, but that a two to three- year period without tillage is not sufficient for populations of anecic earthworms to make a marked contribution to biopore density in the subsoil. The relevance of anecic earthworms for altering physical and chemical properties of biopores during ley cropping still needs further investigation.



## Introduction

Various soil fertility parameters such as soil organic matter content, soil porosity and aggregate size distribution can be influenced by crop rotation design. In organic farming systems, the crop rotation is the most important tool for maintaining soil fertility, managing nutrient fluxes and providing food resources for livestock (Watson et al. 2002). Crop rotations in organic farming systems typically contain ley crops. Soil structure generally improves during the ley phase (Ball et al. 2005) particularly when the ley crops are perennials. As compared with continuous annual cropping, perennial ley crops can reduce the loss of soil carbon and increase the yield of following annual crops in the long term (Persson et al. 2008). Moreover, ley crops such as grass/clover or lucerne can alleviate soil compaction (Hamza and Anderson 2005) and increase the density of biopores (Kautz et al. 2010).

Large sized biopores (diameter >2 mm) in the subsoil can be created by tap roots, which leave voids after their decay or by the burrowing activity of anecic earthworms which take advantage of the temporary lack of tillage in perennial cropping systems. In this paper, the term biopores refers to these large sized, more or less cylindrical voids. Pores of this diameter class are considered to be more or less vertical and continuous and have been reported to increase water infiltration (Edwards et al. 1990; Chan 2004) thus

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**Table 1**
Soil properties at the study site.

| Depth (m) | Horizons (WRB) | Sand (%) | Silt (%) | Clay (%) | Soil textural class | Bulk density ($g\,cm^{-3}$) | pH ($CaCl_2$) | $CaCO_3$ ($g\,kg^{-1}$) | SOC ($g\,kg^{-1}$) | Ntot ($g\,kg^{-1}$) | CEC ($cmolc\,kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0–0.27 | Ap | 8 | 77 | 15 | SiL | 1.29 | 6.5 | <1 | 10.0 | 1.02 | 12.01 |
| 0.27–0.41 | E/B | 5 | 74 | 20 | SiL | 1.32 | 6.9 | <1 | 4.6 | 0.55 | 11.91 |
| 0.41–0.75 | Bt1 | 4 | 69 | 27 | SiCL | 1.42 | 6.9 | <1 | 4.5 | 0.51 | 15.68 |
| 0.75–0.87 | Bt2 | 4 | 65 | 30 | SiCL | 1.52 | 6.9 | <1 | 3.9 | 0.50 | 18.48 |
| 0.87–1.15 | Bt3 | 5 | 70 | 25 | SiL | 1.52 | 7.1 | <1 | 2.5 | 0.34 | 15.49 |
| 1.15–1.27 | Bw | 5 | 72 | 23 | SiL | 1.46 | 7.3 | <1 | 2.6 | 0.34 | 14.35 |
| 1.27–1.40+ | C | 8 | 75 | 13 | SiL | 1.47 | 7.4 | 127 | n.d. | >0 | n.d. |

SOC, soil organic carbon; Ntot, total nitrogen; CEC, cation exchange capacity. The soil refers to a Haplic Luvisol (Hypereutric, Siltic) according to the WRB (IUSS 2006).

**Table 2**
Treatments under study.

| Years | 1. (2007) | 2. (2008) | 3. (2009) | 4. (2010) |
|---|---|---|---|---|
| Lu 1y | * rye | * oats | * lucerne | * winter wheat |
| Lu 2y | * rye | * lucerne | lucerne | * winter wheat |
| Lu 3y | * lucerne | lucerne | lucerne | * winter wheat |
| Chi 1y | * rye | * oats | * chicory | * winter wheat |
| Chi 2y | * rye | * chicory | chicory | * winter wheat |
| Chi 3y | * chicory | chicory | chicory | * winter wheat |
| Fes 1y | * rye | * oats | * fescue | * winter wheat |
| Fes 2y | * rye | * fescue | fescue | * winter wheat |
| Fes 3y | * fescue | fescue | fescue | * winter wheat |

Asterisks (*) indicate inversion tillage events followed by sowing.

counteracting the likelihood of erosion. Furthermore, they are preferential elongation paths for plant roots (Passioura 2002; McKenzie et al. 2009). However, the interactions between root growth and earthworm activity in the process of biopore formation during perennial ley cropping are not well understood. In particular, it is unknown whether anecic earthworms can substantially contribute to biopore formation in comparatively short periods of time during and after perennial ley grown within a rotation. Large sized biopores, which are frequently destroyed in the plough layer can persist for longer periods of time in the subsoil (Edwards et al. 1990). The aim of this study was to quantify the development of the abundance of anecic earthworms and the biopore density during the cultivation of ley crops with contrasting root systems grown for one, two or three years continuously. In this context we aimed to evaluate whether the anecic earthworm *Lumbricus terrestris* preferentially creates new biopores or colonizes abandoned, previously existing biopores under field conditions.

## Materials and methods

### *Site of investigation and experimental design*

The study was undertaken at the Campus Klein Altendorf experimental research station (50°37′ N, 6°59′ E) near Bonn, Germany. The soil is a silty loam soil (WRB: Haplic Luvisol; see Table 1 for more details about the soil properties). Mean annual temperature is 9.6 °C with an average annual precipitation of 625 mm. The investigation was carried out in a field trial with a randomized design with four field replicates and a plot size of 6 × 10 m. We cultivated lucerne 'Planet' (*Medicago sativa* L.), chicory 'Puna' (*Cichorium intybus* L.) and tall fescue 'Hykor' (*Festuca arundinacea* Schreb.) for one, two or three years (2007–2009) continuously, which allowed simultaneous investigation of all treatments (Table 2). Seeding densities for lucerne, chicory and fescue were 25, 5 and 30 kg $ha^{-1}$. Before the ley crops were sown, the soil was annually tilled with a mouldboard plough to 0.3 m soil depth. Consequently, increasing the duration of ley cropping also extended the period without tillage. In each vegetation period, the ley crops were mulched up to four times. Crop residues remained on the soil surface. Before each cut, shoot biomass in each plot was recorded in two subareas (0.5 × 0.5 m each). No pesticides were applied. In spring 2010, the field trial was uniformly ploughed to 0.3 m soil depth and spring wheat was sown.

### *Extraction of anecic earthworms*

Earthworms were extracted from the soil using the mustard extraction method (Gunn 1992). This method has been shown to be efficient for sampling anecic earthworm species (e.g. Lawrence and Bowers 2002). Extractions were carried out during four sampling dates: two in autumn 2009 during ley cropping and two in autumn 2010 after harvest of spring wheat. Each sampling was carried out in four field replicates. For each plot under study, 10 L of tap water containing 85 g mustard (type: 'Düsseldorfer Löwensenf') were poured into a metal frame with a surface area of 0.5 × 0.5 m. Earthworms appearing on the surface within 30 min after application were collected and transferred to water-filled boxes, covered and stored for later identification, counting and weighing. Earthworms were identified according to the key of Christian and Zicsi (1999).

### *Quantification of biopore density*

Biopores newly generated by root growth may not become visible before the decay of the root. Therefore, after the end of the ley cropping stage in spring 2010, we removed the topsoil from 1 $m^2$ subplots in four field replicates, introduced GEOTEX-sheets (1000 g $m^{-2}$) into the soil at 0.4 m depth in order to avoid in growing roots and re-filled the topsoil. After a two-year period, biopores were excavated in spring 2012 by removing the topsoil in sampling areas of 0.25 $m^2$ in size and creating horizontal plane surfaces at a soil depth of 0.4 m. Thereafter, biopores were uncovered from overlying particles with a vacuum cleaner. All apparent biopores – including those filled with roots or soil particles – were visually classified as two size-classes (diameter 2–5 mm and >5 mm). Pores of these size classes can be defined as 'medium macropores' and 'coarse macropores' (Beven and Germann 1982).

### *Recolonization trial*

This experiment was carried out in September 2011 in a three-year continuous grass ley adjacent to the field trial described above. In six field replications the topsoil was removed from circular experimental areas (0.5 m in diameter each). At a soil depth of 0.4 m, a horizontal plane surface was created, biopores were uncovered from overlying particles and the position of each biopore in two size-classes (diameter 2–5 mm and >5 mm) was recorded on a transparency using waterproof markers. Naturally occurring earthworms below 0.4 m depth were removed from the sampling area using the octet method (Thielemann 1986). This method is known to be less efficient for anecic earthworms than the extraction