# Biopore characterization with *in situ* endoscopy: Influence of earthworms on carbon and nitrogen contents

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**Key words:** drilosphere, pore wall structure, earthworms, carbon, nitrogen, subsoil

## Abstract

*Biopores have been shown to be enriched with plant available nutrients as compared to the surrounding bulk soil and therefore are considered hot spots for the nutrient acquisition especially in the otherwise nutrient-poor subsoil. However, depending on their individual biography, i.e. colonization by plant roots or earthworms, different nutrient status can be assumed. In this study, individual biopores were characterized with respect to signs of earthworm passage using in situ endoscopy, a non-destructive technique for display of pore wall characteristics. Subsequent sampling and analysis of biopore linings and blockages revealed that only biopores with visible earthworm coatings had significantly higher C and N contents as compared to the surrounding bulk soil. The results of this study highlight the special role of earthworms for enriching biopores with nutrients and underline the value of biopores for the nutrient acquisition from the subsoil.*

## Introduction

In Organic Agriculture, nutrient management includes strategies for nutrient mobilization from the solid phase of soils. Biopores, generated and used by plant roots and earthworms, provide access to the subsoil. Moreover, they can be considered hot spots for the nutrient acquisition, with favorable biophysical conditions (e.g. less mechanical resistance, higher oxygen content, Stewart et al. 1999) and higher nutrient contents as compared to the surrounding bulk soil (e.g. Graff 1967, Parkin & Berry 1999, Pankhurst et al. 2002). However, depending on their age and history of usage by plant roots and earthworms, individual biopores can differ widely in their physical conditions (Pagenkemper et al. 2014) and in their nutrient status (Kautz et al. in this volume). The objective of the presented study was to examine the effect earthworms have on the nutrient status of biopores, by characterizing individual biopores with respect to a) influence of earthworms and b) carbon and nitrogen contents.

## Material and methods

Biopores were sampled in August 2012 in a field experiment on a Haplic Luvisol (WRB) derived from loess (loamy silt) in Klein-Altendorf near Bonn, Germany (50°37'9''N 6°59'29''E, 9.6 °C mean annual temperature, 625 mm annual rainfall). On the plot sampled, spring wheat (*Triticum aestivum* L.) was grown with precrop tall fescue (*Festuca arundinacea* Schreb.) grown for two years consecutively.

Adjacent to a trench of 150 cm depth, on an area of 50 x 50 cm soil was removed down to a depth of 45 cm, creating a plane horizontal surface. On this area, biopores with a diameter > 5 mm were scraped free using a palette-knife and a vacuum cleaner. Afterwards, a sampling area of 30 x 25 cm was marked. All biopores within this area were investigated with a flexible videoscope (Karl Storz GmbH, Tuttlingen, Germany, outer diameter of 3.8 mm, illuminated with a 150 W cold light projector). The endoscope was introduced only up to 1 cm depth to avoid damaging the pore wall.

To characterize the drilosphere with respect to carbon and nitrogen contents, individual biopore linings and soil material inside the pore volume (biopore blockages) were sampled in 45-55 cm soil depth. All biopores within the sampling area were carefully opened from the side of the trench, using small spoons and scrapers. Throughout the sampling area, six bulk soil samples were additionally collected. For all samples, total C and N contents were determined with elementary analysis (Euro EA 3000, HEKAtech, Germany).

Data were submitted to ANOVA followed by Tukey-test at $\alpha = 0.05$.

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## Results

Endoscopy images enabled detection of earthworm cast both in biopore linings (Figure 1) and blockages (Figure 2).

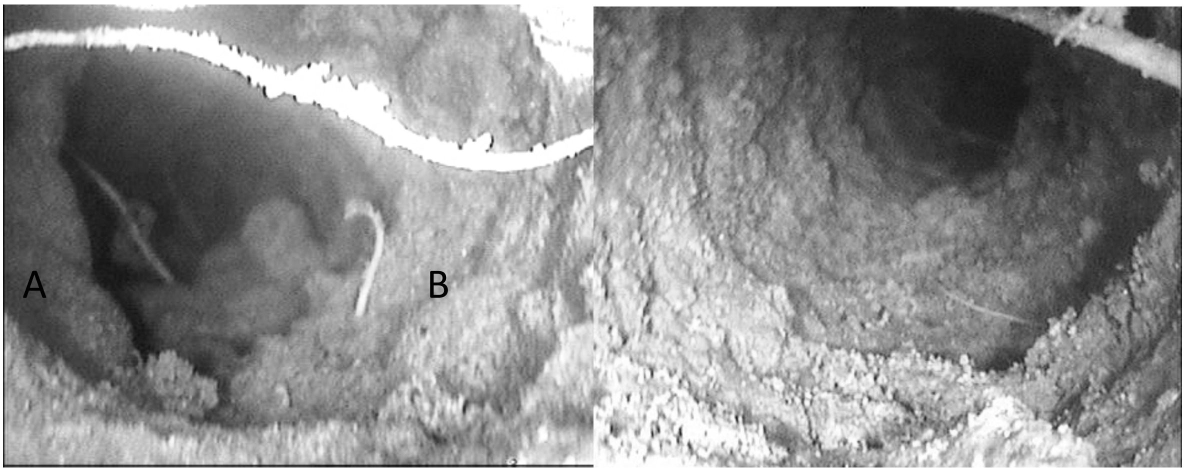


**Figure 1. Biopore lined with earthworm cast (A) and biopore lined with material not clearly originating from earthworm cast (B).**

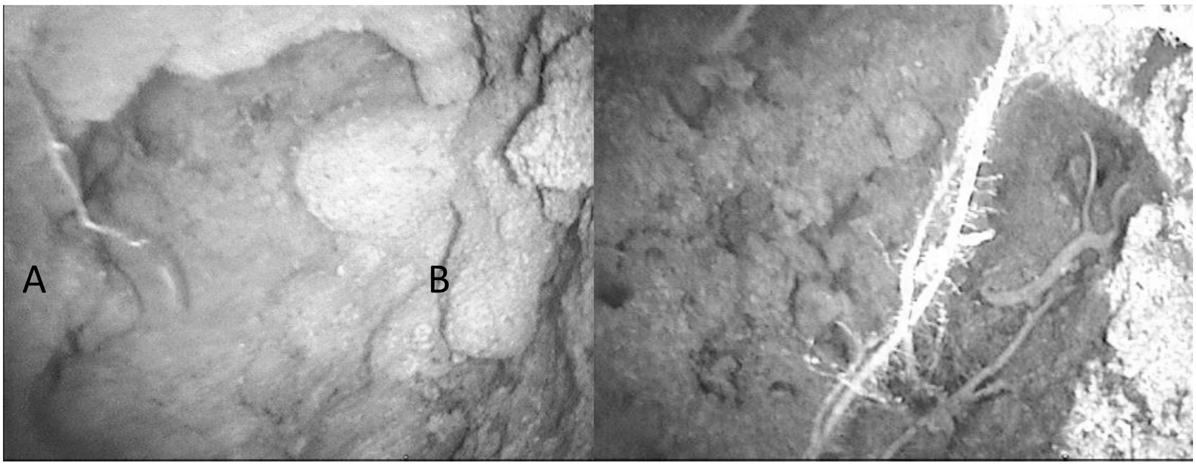


**Figure 2. Biopore blocked with earthworm cast (A) and biopore blocked with material not clearly originating from earthworm cast (B).**

Within the sampling area, 17 biopores were photographed with the endoscope and sampled. Out of these, 10 were characterized as clearly coated with earthworm cast (biopore+EW), and 7 were characterized as not clearly coated with earthworm cast (biopore-EW). Both for C- and N-content, only the pores with clearly visible earthworm coatings showed significantly higher values as compared to the bulk soil (Figure 3). Biopores-EW ranged for all three parameters in between biopores+EW and the bulk soil.

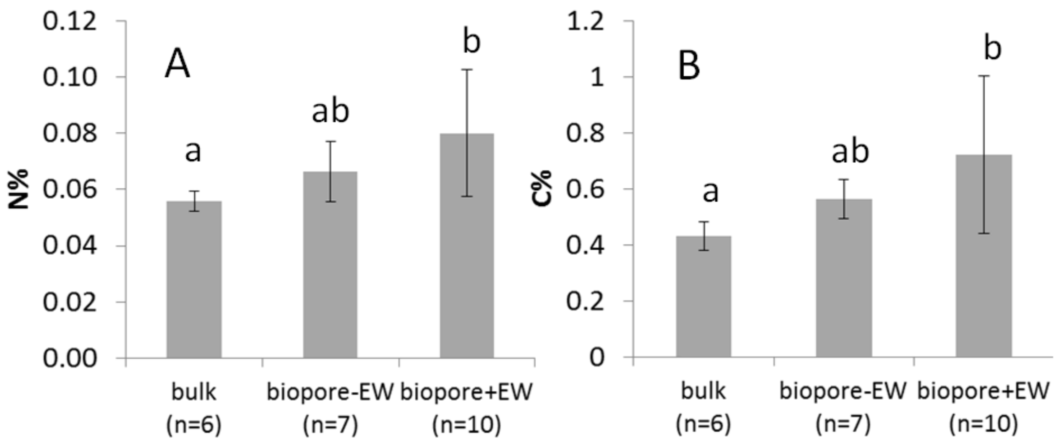


**Figure 3. N-contents (A) and C-contents (B) in bulk soil and biopores according to endoscopy images. Biopore+EW: clearly coated with earthworm cast, biopore-EW: not clearly coated with earthworm cast. Different letters indicate significant differences (Tukey-test, α = 0.05). Error bars represent standard deviation.**

## Discussion

At the site under study, two years of continuous cultivation of tall fescue as compared to one year resulted in a significant increase in the population of anecic earthworms (Kautz et al. 2011). However, in the presented study recent earthworm coatings were visible only in about 60% of all biopores investigated. The site under study is conventionally managed. It is possible that under organic management, with longer periods of soil rest e.g. under grass clover and more organic residues on the soil surface, the population of earthworms would increase even more on the long run, with consequently more pores exhibiting signs of earthworm influence and possibly also higher nutrient contents of pore walls and blockages.

The variation of C- and N-contents in biopores+EW was comparatively high and there was no significant difference of C- and N-contents between biopores+EW and biopores-EW. Obviously, the earthworm casts in biopores+EW were heterogeneously distributed over the pore wall or pores with older earthworm casts, already depleted in C and N, were classified as biopore+EW.

Earthworms can modify biopore properties considerably. After earthworm passage, Pagenkemper et al. (2014) detected changes in the physical properties of biopores, e.g. increasing path lengths, widths and connectivity, with possible implications for root growth in biopores and consequently nutrient uptake. Kautz et al. (2014) found that at the site under study, earthworms prefer colonizing existing biopores – thereby increasing nutrient contents of pore walls and blockages, as shown in our study.

Nutrient contents of biopore walls are relevant for nutrient acquisition from the subsoil: Athmann et al. (2013) showed that roots in biopores establish contact to the pore wall in most cases, i.e. that they can profit from nutrient enrichment of biopore linings and blockages. Furthermore, roots show a preference for nutrient rich pores (Kautz et al. in this volume).

## Conclusions

The results of this study suggest that promotion of anecic earthworms contributes to enrich biopores with nutrients. Apart from the total number of biopores, the properties of individual biopores are presumably also relevant for the nutrient acquisition. Therefore, management strategies in organic farming should focus on increasing both biopore quantity (e.g. by cultivation of taprooted precrops) and quality, via promotion of anecic earthworms. More research is needed on usage of biopores by plant roots and earthworms, including nutrient mobilization and acquisition processes inside and around biopores and interaction of plant roots and earthworms.

## Acknowledgements

This study was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft – DFG) within the framework of the research unit DFG FOR 1320. Details of the field trials potentially open for external researchers: www.for1320.uni-bonn.de.

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