



UMEÅ UNIVERSITY

# **Quantifying changes in soil bioporosity in subarctic soils after earthworm invasions**

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## Abstract

Pores provide important hotspots for chemical and biological processes in soils. Earthworm burrows affect the macropore structure and their actions may create new preferential pathways for water and gas flow within soils. This, in turn, indirectly affect plants, nutrient cycling, hydraulic conductivity, gas exchange, and soil organisms. While the effects of invasive earthworms on soil properties has been well-documented in temperate and boreal ecosystems, we know little how these organism may affect tundra soils. In this study, I assessed how the three-dimensional network of soil-macropores are affected by earthworm species (*Aporrectodea* sp. and *Lumbricus* sp). I hypothesized: i) that earthworms increase the frequency of macropores with a likely biological origin (biopores); ii) effects of biopores are dependent on tundra vegetation type (meadow or heath); and iii) the macropore network properties are altered by earthworms. The hypotheses were tested using a common garden experiment with 48 mesocosms. The pore structure of each mesocosm was analyzed using X-ray CT tomography. I found that biopores increased in the tundra from on  $0.05 \pm 0.01$  % (mean  $\pm$  standard deviation) in the control to about  $0.59 \pm 0.07$  % in the earthworm treatments. However, in contrast to my second hypothesis, I found no vegetation dependent effect. Interestingly, I found that earthworms decreased the complexity and directionality of macropores. My findings strongly indicate that burrowing can severely impact the pore properties of previously uninhabited subarctic soils.

**Key words: Biopores, macropores, geoengineering earthworms, X-ray tomography**



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# 1 Introduction

Pores are both products and drivers of a variety of abiotic and biotic soil processes (Kravchenko and Guber 2017). Pore spaces are biological hotspots that are often more chemically reactive than the surrounding bulk soil (Jarvis 2007). Further, microbial decomposition of soil organic matter depends on organic matter accessibility, which in turn partially depend on soil pore space and connectivity (Dungait et al 2012). In other words, understanding soil pore formation and changes over time is crucial for predicting how soils respond to environmental change.

It is well-established that earthworms are ecosystem engineers due to the profound impact on their environment and soil pore system (Capowiez, Pierret and Moran 2003). Earthworms burrow through soils creating macropores that generally increase the overall soil porosity and alter pore structures (Gorres and Amador 2010). The transfer of liquid and gaseous content through the soil profile is directly related to the geometry of the soil pore network (Vogel and Roth 2001). Earthworms subsequently modify soil water infiltration, gas diffusion, and the transfer of nutrients through the soil (Capowiez, Pierret and Moran 2003). Soil CO<sub>2</sub> and N<sub>2</sub>O production and emissions are indirectly related to earthworm bioturbation by mixing soil layers, changing gas diffusivity and soil moisture dynamics (Lubbers et al 2013). Furthermore, bioturbation modifies the distribution of soil organic matter, which could potentially affect carbon cycling by disconnecting organic substrates from the pore space in which microbes move (i.e. aggregates) or by enhancing the physical interaction between organic and mineral matter (Lyttle et al 2015). Meta-analysis found that earthworms increased soil CO<sub>2</sub> and N<sub>2</sub>O emissions by 33% and 42% respectively, but it has been hypothesized that the increased incorporation of substrates and thus, reduced availability for microbes, can over time reduce CO<sub>2</sub> emissions (Lubbers et al 2013). Nevertheless, studies assessing how aggregates formed by earthworms affect interactions between minerals and organic matter are mainly based on sieved soil samples. It has recently been shown that these classic methods are flawed and that aggregates are an artificial product depending on the energy used during the separation step (Amézketa 1999). Therefore, studies has suggested that the way forward for soil studies is to focus on soil pores (the inverse of aggregates) of intact soil cores rather than strongly processed soil samples (Baveye 2020).

Despite it being reported of the beneficial functions earthworms perform in agroecosystems by increasing primary production, reducing runoff and improving soil structure and stability, earthworms pose a serious threat to previously uninhabited ecosystems (van Groenigen et al 2014, Bertrand et al 2015; Wackett et al 2018); Frelich et al 2019). Natural ecosystems that were previously uninhabited by earthworms seem to experience mainly negative effects following earthworm invasions due to the severe impact they have on soil morphology as well as entire ecosystems (Bohlen et al 2004; Migge-Kleian et al 2006; Wackett et al 2018). Invasive earthworms can cause changes in soil pore systems (Alban and Berry 1994; Hale et al 2005), carbon cycling (Lyttle et al 2015), inorganic nutrient availability (Resner et al 2014) and understory plant communities (Hale et al 2006). It has also been suggested that a decline in soil fauna as a response to earthworm invasion may have strong negative impacts on indigenous plant groups (Migge-Kleian et al 2006).

The alpine region of Fennoscandia is one of the last European regions where earthworm populations are still very limited (Wackett et al 2018). In this latter study, earthworms were shown to disperse mainly through anthropogenic activity, such as agriculture, fishing and gardening. An invasion of non-native earthworms could potentially cause changes to the soil pore systems of these sensitive environments. It has however not yet been observed if earthworms are potent enough to change the Arctic soil pore system. Nevertheless, when introduced by human activity, earthworm species such as *Lumbricus* species and *Aporrectodea* species have been found to be able to survive in the arctic biome (Blume-Werry et al 2020). These earthworms are active within the organic soil horizons and the upper edge

(about 30 cm) of the mineral soil (*Lumbricus rubellus*), together with species that burrows deeply into mineral soil (anecic *Lumbricus terrestris* and endogeic *Aporrectodea* species) (Blume-Werry et al. 2020). When introduced, these species are known to be responsible for considerable ecosystem changes and are referred to as geoengineering species (Hale, Freilich and Reich 2006; Resner et al 2014; Wackett et al 2018). Once established, they are difficult to eradicate without major environmental impacts: hence, it seems urgent to quantify their eventual impacts on tundra systems before they are allowed to become more widely distributed within the Arctic.

Survival of earthworms largely depends on the physical and chemical properties of soils, which includes soil pH, moisture and soil organic matter quality (Singh et al 2020); hence, it is not self-evident that earthworms can affect pore systems in cold and more acidic arctic soil as typically observed for the more studied agricultural soils. For example, the vulnerability of arctic soil and its response to earthworms seems likely to vary depending on earthworm survivability in different vegetation types. In arctic and alpine regions, tundra heath and meadow are co-dominant vegetation types that occurs over large areas (Walker et al 2005; Sundqvist et al 2014). In comparison, meadow is more nutrient-rich, has consistently a higher pH and is dominated by herbaceous species whereas heath is dominated by woody dwarf shrub species (Björk et al 2006; Sundqvist et al 2014). Indeed, this habitat seems more preferential for earthworms than more acidic and nutrient poor heath soils.

But what pores are expected to be affected by earthworms? Macropores (75  $\mu\text{m}$  - >5 mm) created by biological activity, biopores, are formed by the movement of roots and macrofauna, i.e. primarily by earthworm burrows (Kirkham 2005; Zhang et al 2018). They are typically continuous cylindrical or tubular-shaped voids and are generally vertically oriented and can extend along the soil profile (Kautz 2014; Zhang et al 2018; Landl et al 2019). Three-dimensional properties, such as the size, shape, structure, distribution and connectivity of biopores and non-biological pores can be accurately interpreted and studied at a macro- metric scale in a non-invasive and non-destructive way using X-ray computed tomography and image analysis (Taina, Heck and Elliot 2008). The technique allows for visualization of soil profiles and greater understanding of the three-dimensional organization of soils. Using this technique, changes in soil morphology as a response to earthworm introduction can be seen.

In this study, intact soil cores from a tundra mesocosm experiment (located in Abisko, northern Sweden) were analyzed using X-ray tomography. Three-dimensional analysis of the pores in heath and meadow mesocosm subjected to earthworm treatments (N=24) were compared with control soil samples (N=24) without treatment. I hypothesized that: (1) earthworms increase the frequency of bioporosity in the tundra. (2) Effects on biopores are dependent on vegetation type and (3) earthworm treatment cause changes in macropore network characteristics.

## **2 Materials and Method**

### **2.1 Mesocosm experiment**

Earthworm treated mesocosms used in this analysis is based on a mesocosm experiment which is described in detail in Blume-Werry et al (2020). The mesocosms were constructed using soil monoliths taken from natural tundra vegetation. The monoliths were collected in the Kärkevagge valley (68°24'36'' N; 18°19'11'' E) at 700m elevation. 48 soil mesocosms were installed in the autumn of 2013 from two vegetation types, heath and meadow. Patches of land that represented each vegetation type were selected.



## 2.2 Earthworm treatment

*Aporrectodea* species and *Lumbricus* species were added to the mesocosms on the 9<sup>th</sup> of June 2017. Half of the total amount of mesocosms were designated earthworm-free controls. To compensate for high mortality rates during winter frost, earthworm addition was repeated every spring until the termination of the experiment in 2020. The rationale for this was to ensure a living population in each earthworm mesocosm as the 30 cm deep mesocosm makes it impossible for the earthworms to retreat to frost-free soil and full eradication in the mesocosms is therefore expected during the winter. In these mesocosms, 48 intact cores with a diameter of 10 centimeter and a depth of approximately 20 cm were taken. The cores were wrapped in cellophane and frozen to preserve the soil structure for scanning.

## 2.3 X-ray computed tomography

Cores were scanned using a GE phoenix v|tome|x m X-ray tomograph. The scans were carried out at the Swedish University of Agricultural Sciences in Uppsala. Insulation to preserve frozen cores were added during scanning. A plastic rod covering the length of the sample was put inside the insulation for future calibration of the images. The cores were scanned horizontally at energies of 150 kV and with a current of 570  $\mu$ A. Each scan consisted of a collection of 1800 images. Image averaging was set to 2 and skip 1. The timing of the detector was set to 250ms. The voxel resolution was set to 120 $\mu$ m. The total scan time was approximately 21 minutes per scan.

## 2.4 Image Processing

3D reconstruction, processing and analysis was carried out using the ImageJ/Fiji platform. The Tubeness plugin was used to extract biopores and the SoilJ plugin (Koestel 2018) was used for biopore analysis. Image resolution was lowered to 240  $\mu$ m to decrease computational processing time. A 3D median filter with a radius of 2 was added to reduce image noise. The soil columns were rotated to upright positions. Due to differences in illumination during scanning, gray-scale values were calibrated to ensure that identical densities across the columns correspond to same gray-scale values. Calibration was carried out using the plastic rod and air inside the column, which were assumed to have homogeneous density and could thus be used to reduce the effect of illumination-related errors. A circular region of interest (60 mm diameter) at the center of each column was cropped so that a horizontal cross-section area was identical for all columns. However, the soil columns had a large amount of variation in the depth of the organic horizon. As a consequence, the organic layer was removed for all columns which reduced computational time and facilitated biopore analysis.

Further, three-dimensional binary images of the soil columns were obtained by thresholding segmentation, partitioning the data into two categories based on gray-scale values to extract pores from material. Since pores share similar gray-scale values in the calibrated images they can be separated from matter of higher density (appendix 1).

Following that pores were separated from the soil matrix, the biopores were extracted using the Tubeness plugin in Fiji, ImageJ which distinguishes tubular shapes from irregular shapes (Lucas et al 2019). The tubeness threshold value was set to 60. Finally, quantitative image data was extracted using the SoilJ plugin which automates the processing steps for extracting data out of three-dimensional X-ray images (Koestel 2018).

## 2.5 Pore network measures

Biopore network characteristics used in this analysis are shown in Table 1. An indicator for the global pore connectivity is the connection probability, defined by Renard and Allard (2013). This measure covers the vertical and horizontal connectivity between pore clusters and is positively correlated with soil aeration (Koestel and Schlüter 2019). Connectivity in general is thought to also influence soil hydraulic conductivity, gas exchange and water retention (Lucas et al 2018). Connection probability was used to compare macropore network

connectivity between vegetation types and treatments.

The geometry of soil pores has a strong impact on water availability and retention, and the rate of water and solute transport in soil (Payne 1988). The fractal dimension is a measure describing an objects complexity (Perret, Prasher and Kacimov 2003). In this study, it was used to describe the heterogeneity of the macropore network.

The anisotropy, i.e., a measure on how different the pore properties are in different directions and opposed to isotropy, significantly affect the transport of liquid and gaseous content (Berisso et al 2013). The relevance of this measure served as a rationale for including it in my analysis of how earthworms affect macropore network characteristics (hypothesis 3). Anisotropy was calculated using the total number of crossings in the X, Y and Z-direction. A crossing means a change in value (i.e. a change from white to black, or vice versa, in the binary images used for biopore analysis) occurs. The anisotropy was determined by the expression:

$$\text{Eq 1.} \quad (C_{max} - C_{min}) / (C_{max} + C_{min})$$

In which Cmax and Cmin stands for the direction (X, Y or Z) with the maximum and minimum number of crossings.

Table 1. Measures used to describe changes to the macropore network as a response to earthworm treatment.

Measure (unit)	Description
Macroporosity (%)	Volume of total pores divided by total volume multiplied by a factor of 100 to express macroporosity as a percent.
Bioporosity (%)	Volume of biopores divided by total volume multiplied by a factor of 100 to express bioporosity as a percent.
Anisotropy	Measure indicating if geometric properties depend on direction. Values range from 0 to 1. A value close to 0 indicates isotropic conditions (i.e. no preferential direction)
Connection probability	Measure of global connectivity. A value of 1 means all macropores are connected whereas a value of 0 means no pore clusters or single macropores are connected
Fractal dimension	Measure of complexity. The value of the fractal dimension increases when shapes are more complex.

## 2.6 Statistical Analysis

Units of biopore measures were converted from voxels to mm. Bioporosity was calculated using the total volume of biopores divided by total sample volume. Additional biopore measures used in the statistical analysis are shown in table 1. Two-way analysis of variation (2-way ANOVA) was carried out in SPSS Statistics (27.0.0.0) to test each hypothesis and biopore measures between vegetation types and treatments. The residuals of the ANOVA model were inspected visually and judged to fulfill the normal distribution criteria. Plotting was done using Excel 2016.

## 3 Results

Cores were scanned successfully and images of all 48 cores from the mesocosm were retrieved. Bioporosity (>240 µm) was found to be higher in treatments (F=63.9, p<0.000) compared to controls which supports the 1<sup>st</sup> hypothesis (Figure 1a). Mean bioporosity (%) for earthworm-free controls and earthworm treated columns was 0.05 ± 0.01 (mean ± standard deviation) and 0.59 ± 0.07 respectively. In addition, total macroporosity (%) increased as a response to earthworm treatment (F=26.4, p<0.000). Mean macroporosity (%) was 2.7 ± 0.4

in controls and  $6.4 \pm 0.6$  in earthworm treatments. The 2<sup>nd</sup> hypothesis was rejected as no difference in bioporosity was found between vegetation types ( $F=0.36$ ,  $p=0.554$ ) (Figure 1b). Mean bioporosity for meadow and heath mesocosms was  $0.30 \pm 0.06\%$  and  $0.34 \pm 0.09\%$  respectively. Similarly, no difference was found in total macroporosity between vegetation types ( $F=0.27$ ,  $p=0.608$ ). No measures showed an interaction effect between treatments and vegetation type.

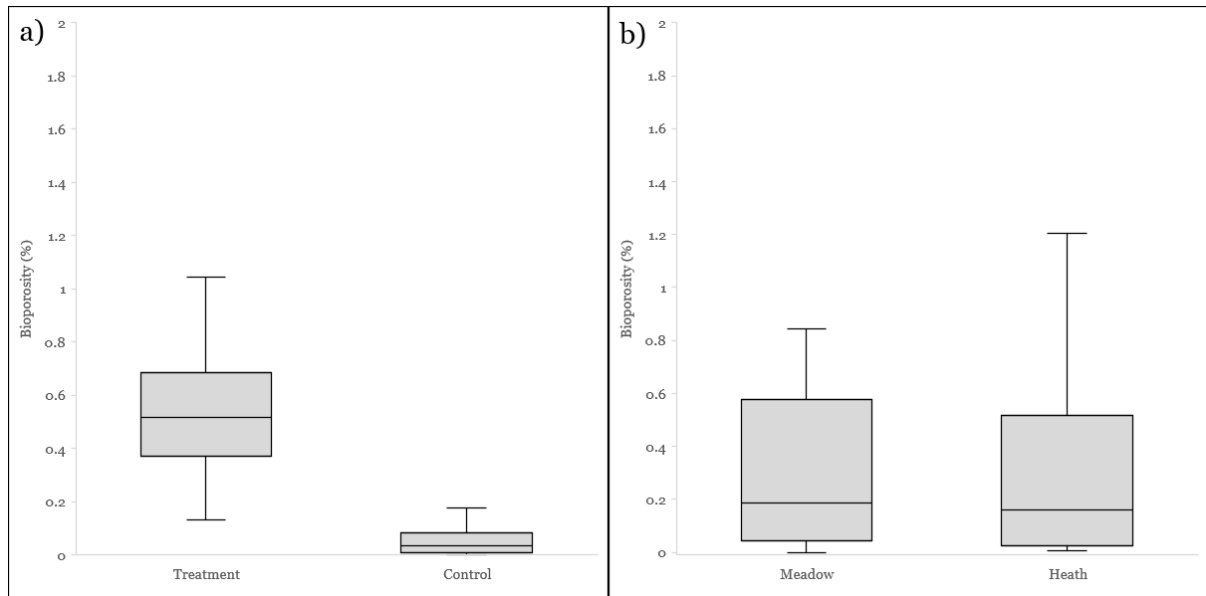


Figure 1. Bioporosity (%) between treatments (a), and vegetation type (b). A significant increase in bioporosity was found in earthworm-treated mesocosms ( $F=63.9$ ,  $p<0.000$ ). No significant difference was found between vegetation types. Whisker bars show minimum and maximum values.

The degree of anisotropy was very low both between control and treatments and between heath and meadow (close to isotropic conditions). However, as shown in figure 2a, earthworm treatment reduced macropore network anisotropy ( $F=22.4$ ,  $p<0.000$ ). Mean anisotropy for treatment and control was  $0.029 \pm 0.005$  and  $0.066 \pm 0.006$  respectively. No difference was found between vegetation types ( $F=0.06$ ,  $p=0.804$ ). The global macropore connectivity measure showed a large variation between mesocosms, with values ranging from 0.017-0.927. Global connectivity was not affected by neither vegetation type ( $F=0.79$ ,  $p=0.377$ ) nor treatment ( $F=0.03$ ,  $p=0.869$ ). The complexity of the macropore network (fractal dimension) was significantly lower in earthworm treatments ( $F=13.01$ ,  $p=0.001$ ) (figure 2b). No difference was found between heath and meadow ( $F=0.01$ ,  $p=0.919$ ). Neither of these measures (anisotropy, connectivity, and fractal dimension) showed an interaction effect between vegetation type and treatment.

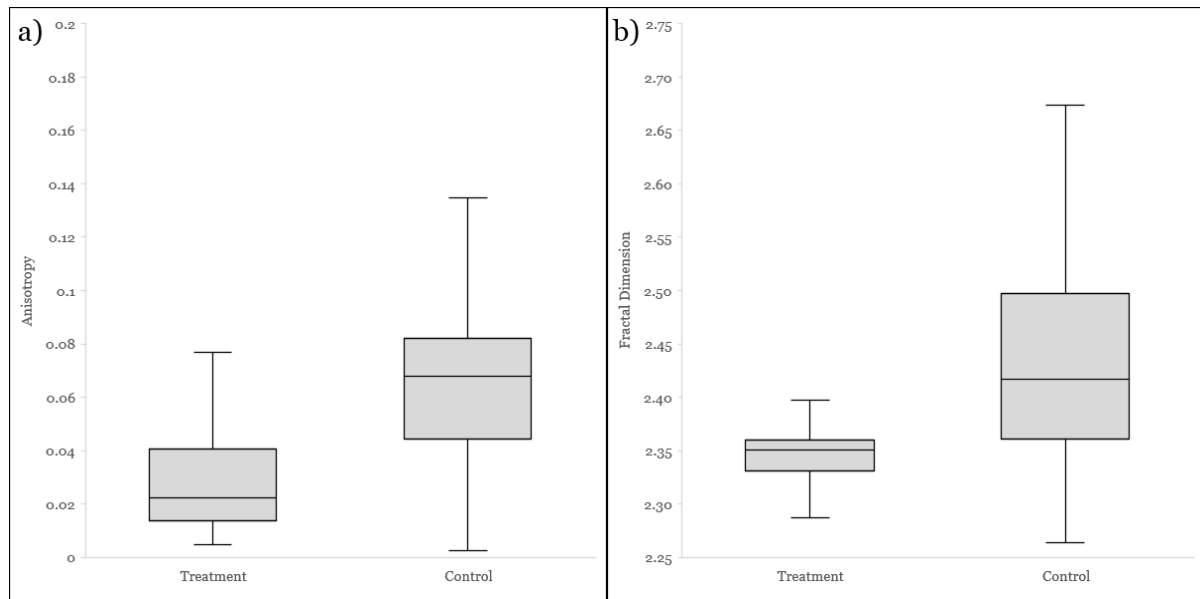


Figure 2. Anisotropy (2a) and the fractal dimension (2b) between treatments and controls. The anisotropy analysis showed that control mesocosms had a larger degree of anisotropy ( $F=22.4$ ,  $p < 0.000$ ). Earthworm treatments significantly decreased the complexity of the macropore network ( $F = 13.0$ ,  $p 0.001$ ). Whisker bars show minimum and maximum values.

## 4 Discussion

As my results indicate, the first hypothesis was valid. Earthworm treatment significantly increased bioporosity. Indeed, this is not a surprise as earthworm burrows are by definition biopores, but the result highlight that the applied method was able to correctly separate pores made by biota from pores made by abiotic processes. However, despite the reported differences in pH and nutrient availability between heath and meadow soils (Björk et al 2006), I found no difference in biopore frequencies between these two vegetation types. I anticipated that these differences would affect bioporosity (hypothesis 2), and that the more nutrient-rich and less acidic environment in the meadow would benefit earthworms and thus increase activity and biopore formation. Nevertheless, my second hypothesis was supported by data. Interestingly, this finding indicates that there is no differences in earthworm activity between the studied vegetation types and that earthworms' are as active in the heath system as in the meadow. This is an important finding as it suggests that earthworms can expand their habitats into shrub dominated tundra that cover large parts of the Arctic (Walker et al 2005).

Further, the decision not to retain the organic horizon might have affected the results. The depth of the organic horizon largely varied between mesocosms. However, during image processing, it was clear that meadow mesocosms generally had a deeper O-horizon than heath mesocosms. Generally speaking, most activity occur in the topsoil and it is possible that this might have decreased variation in measures between each vegetation type. For instance, pH, nutrient availability and organic content in the organic horizon may be lower than in the mineral soil, which could have led to different results.

One intriguing finding is that biopores were present in all controls. These biopores may originate from native litter-dwelling (epigeic) earthworm species which have been observed in similar conditions, as reported by Wackett et al (2018). Epigeic earthworm species have been previously found to burrow within the upper 20 cm of the soil (Shuster, Subler and McCoy 2002). The organic horizon was not included in this analysis but these epigeic earthworms could potentially have formed, at least partly, these biopores. Besides earthworms, it is likely that some biopores originate from plant roots which, similar to

earthworms, create continuous tube-shaped biopores. However, differences in plant-communities between vegetation types did not cause a difference in bioporosity. A possible explanation is that if biopores in controls were predominantly created by roots, a more distinct difference might have been seen between vegetation types due to differences in root systems.

Mean macroporosity more than doubled after treatment, which is a strong effect of earthworms. This is in agreement with the results of Lamandé et al (2011). They found that macropore density was directly related to anecic earthworm density. This increase in macroporosity indicate that invasive earthworms may have a potent influence on arctic soils as they can increase soil macro volume and subsequently a variety of processes, such as soil water conductivity, gas exchange and microbial decomposition (Vogel and Roth 2001; Dungait et al 2012).

A higher degree of anisotropy was found in controls. Consequently, earthworm burrows did not exhibit any preferred direction. I assumed that earthworm burrows may follow a directional pattern which would increase anisotropy. Yet again, I was wrong. A possible explanation why anisotropy decreased is that the anecic and endogeic earthworm species (*Lumbricus* and *Aporrectodea* species) burrow both vertically and horizontally, as has been previously suggested (Wackett et al 2018). Further, earthworm activity may have compacted or decompacted the soil which could have had an impact on the anisotropy on the entire macropore network. However, mean anisotropy was very low in both treatments and controls and, in several cases, values were close to zero, indicating isotropic conditions (Figure 2a).

Macropore network complexity was lower in earthworm treatments (figure 2b), suggesting that my third hypothesis was valid: earthworms are potent enough to change pore network properties. In short, a lower network complexity indicates that earthworm burrows are more homogeneous than macropores in the control. Similar results were reported in Larsbo et al (2014) in which they found that large macro-porosities are spatially more homogeneously arranged. To some extent, a more homogeneous pore structure, with continuous biopores of low complexity, might affect the water and solute transport in the soil. Furthermore, a reduction in complexity might affect the specific surface area that greatly influences water retention (de Jong et al 1996) and the sorption of inorganic and organic substances (Scheidegger and Sparks 1996). Earthworms have been previously reported to increase nitrogen leaching in agricultural systems (Domínguez, Bohlen and Parmelee 2004; Subler, Baranski and Edwards 1997). Hence, it is possible that earthworms in subarctic systems may enhance leaching and thus alter nutrient dynamics, retention, and water and solute transport by decreasing the complexity of soil pore structure.

In contrary to the 3<sup>rd</sup> hypothesis, no affect in connectivity on the pore network was found. Since these earthworms burrow deeply into mineral soil, it was thought that the connectivity between vertical and horizontal macropore clusters would increase. The connectivity between macropores did not increase even though the total amount of macropores increased. An interesting follow-up analysis would be to study if earthworms caused an increase in macropore connection to the topsoil, which would increase soil aeration (Koestel and Schlüter 2019). However, such an analysis was deemed inappropriate since the organic horizon was not analyzed in this study.

In a similar environment, invasive earthworms have recently been shown to contribute to higher nitrogen levels which may lead to ecosystems changes as these systems are often nitrogen limited (Lubbers et al 2013; Wackett et al 2018). However, the effects of geoenvironmental earthworms have mainly been reported from North American temperate and boreal forests where they are dispersing and invading previously uninhabited areas through anthropogenic activity (Craven et al 2016). Apart from geomorphological changes, earthworms influenced plant species diversity in a negative way (Craven et al 2016) and caused dramatic changes in biogeochemical cycling and hydrology (Larson 2009).

Furthermore, the latter study concluded that increased drainage due to earthworm burrows is thought to increase the frequency in occurrence of drought. As temperature is increasing twice as fast in the arctic compared to global averages and the intensity and frequency of future droughts increase, drainage might accelerate loss of drought-sensitive organisms (Larson 2009; IPCC 2013; Cohen et al 2014;). Furthermore, subarctic and Arctic ecosystems store large quantities of organic matter close to the surface and frozen deep underground (Schuur et al 2015). By mixing the soil and increasing nitrification and denitrification rates, they are threatening to increase the release of greenhouse gases in Arctic soils (Lubbers et al 2013). My data indicate that pores and mixing occur to at least 20-30 cm depth in the mesocosm and thus, earthworm mixing could affect the C and N pools stored at least down to this depth.

My study suggest that that earthworms can re-shape pore properties of heath and meadow soils, two dominant vegetation types in arctic ecosystems. It is very clear that earthworms can cause substantial changes arctic soil morphology, which may have consequences for ecosystem functions. The observed 'worm-induced' changes largely resembles effects reported from North American forests. In other words, earthworms seems to do the same things in Arctic soils as in soils from warmer biomes. However, future research should continue to address if earthworms are limited by arctic soil conditions and, if they impact carbon pools, which may induce substantial greenhouse gas emissions that could further boost the ongoing climate change.

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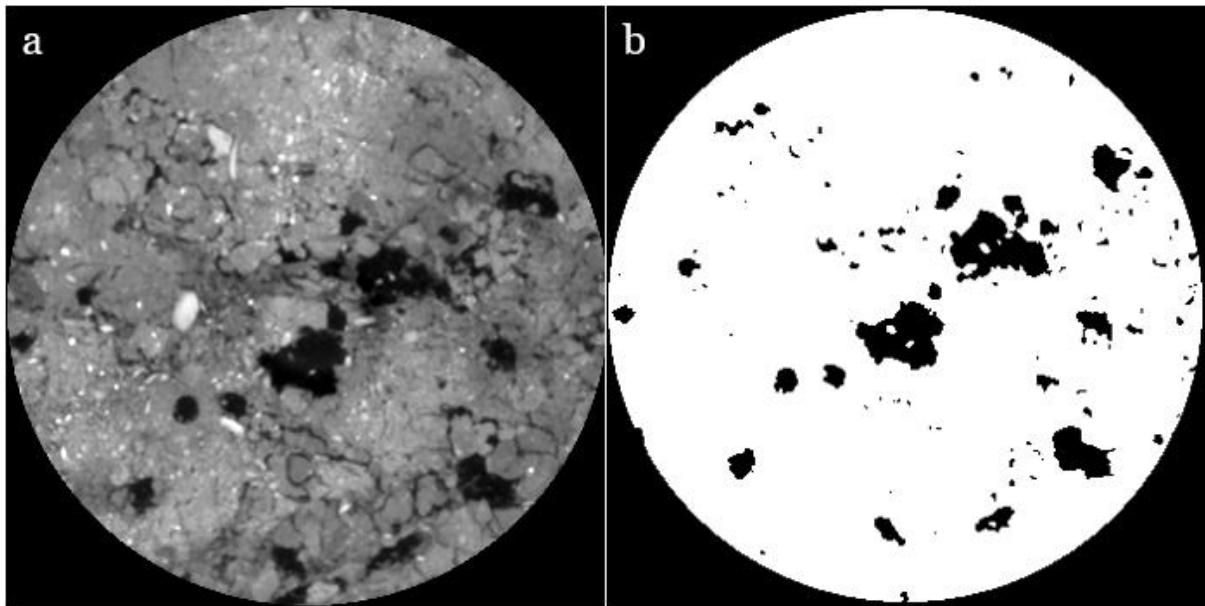
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## 7 Appendix



Appendix 1. Visual example of a 2-dimensional cross-section before (1a) and after (1b) thresholding segmentation. Based on gray-scale values, the data is partitioned into two categories where pores are extracted from material into binary images.