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Partitioning of habitable pore space in earthworm burrows

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Abstract: Earthworms affect macro-pore structure of soils. However, some studies suggest that earthworm burrow walls and casts themselves differ greatly in structure from surrounding soils, potentially creating habitat for microbivorous nematodes which accelerate the decomposition and C and N mineralization. In this study aggregates were sampled from the burrow walls of the anecic earthworm *Lumbricus terrestris* and bulk soil (not altered by earthworms) from mesocosm incubated in the lab for 0, 1, 3, 5 and 16 weeks. Pore volumes and pore sizes were measured in triplicate with Mercury Intrusion Porosimetry (MIP). This method is well suited to establish pore size structure in the context of habitat, because it measures the stepwise intrusion of mercury from the outside of the aggregate into ever smaller pores. The progress of mercury into the aggregate interior thus resembles potential paths of a nematode into accessible habitable pore spaces residing in an aggregate. Total specific pore volume, V_s, varied between 0.13 and 0.18 mL/g and increased from 3 to 16 weeks in both burrow and bulk soil. Differences between total V_s of bulk and burrow samples were not significant on any sampling date. However, differences were significant for pore size fractions at the scale of nematode body diameter.

Key words: Ecology, earthworm burrows, habitable pore space, mercury porosimetry.

The permanent, vertical burrows of anecic earthworms are foci of increased biological activity (Devliegher and Verstraten, 1997; Gorres et al. 1997; Brown et al., 1995) with nematodes and protozoa abundances exceeding those in surrounding soils several fold (Tiunov et al., 2001; Savin et al., 2004). Increased nutrient and carbon mineralization rates frequently observed in burrow soils may be a result of microbivory by these soil animals. Evidence for this is the co-occurrence of greater nematode abundance with accelerated mineralization rates (Gorres et al., 1997; Savin et al., 2004). The increase in activity may be due to resource enrichment through the translocation of detrital resources by earthworms from the soil surface.

It is known that earthworms have a profound effect on soil structure at the macro-scale as they excavate macropores and thus change the overall porosity of the soil. These macro-pores improve the diffusion of O_2 into the soil and thus enhance decomposition rates (Devliegher and Verstraten, 1993; Kretzschmar and Monestiez, 1992). However, differences between aggregate structure (Jongmans et al. 2001; Bossuyt et al., 2006) and pore structure (Gorres et al., 2001) of earthworm cast and non-cast (bulk) soils were also observed at smaller scales. For example, in a silt loam, burrows and middens were shown to have less specific pore volume (total pore volume per unit mass of oven dry soil) than surrounding soils (Gorres et al., 2001).

Several hypotheses link the pore structure of soils to the ability of nematodes to actively participate in decomposition by microbivory. The habitable pore space hypothesis (Elliott et al., 1980) states that soil animals can only access pore spaces with pore sizes greater than their body diameter. Postma and vanVeen (1990) distinguish between four pore size ranges for bacteria: total pore volume, accessible volume, habitable volume and protected pore volume. They suggest that bacterial cells are protected from predation by protozoa in soil pores less than 3 μm in diameter. Protection of microorganisms from grazing by nematodes may occur at larger pore size thresholds (Hassink, 1993). Pore structure of earthworm burrows and middens can have a profound effect on local decomposition and mineralization rates. In addition, pore structure may also affect the ability of substrate to diffuse from earthworm burrows so that soils beyond the burrow might benefit from the increased transformation rates observed in the burrows (Amador et al., 2006).

The interpretation of pore size distributions in terms of habitable pore space is not as straight forward as some of the literature suggests. The capillary bundle model conceptualizes soil pore volume as a composite of cylindrical capillaries which relates volumes to pore diameters with simple geometric considerations (Jury, 1991). Cylindrical pore models have been successfully used to predict the transmission of water through the soil. Yet, soil pores are interconnected, tortuous and have variable diameters along their length. Thus, cylindrical models do not suitably represent habitat of micro- and mesofauna.

Several authors have investigated aggregates as habitat for protozoa or nematodes (Hattori, 1994; Sano and Nakasono, 1997; Queneheve and Chotte, 2001). Aggregate based micro-habitat concepts may be represented by so-called Mobile-Immobile Water Models (MIM, van Genuchten and Wierenga, 1976; Nkedi-Kizza et al, 1983). MIMs better predict solute retardation during transport through the soil than the cylindrical model because they allow the retention of solute in the immobile water phase when soils are sufficiently dry (Addiscott, 1977; Rao et al, 1983). An exchange between mobile and immobile water during saturation allows diffusion from the immobile into mobile phase.

Capillary-based concepts of drying predict that pores drain from large to small, excluding soil animals with bodies wider than the largest water-filled cylindrical capillary. This concept, the "exclusion hypothesis" (Elliott et al. 1982, Hassink et al. 1993), is at odds with observations. Neher et al. (1999) and Yeates et al. (2002) showed that nematode populations grew at moistures levels where they theoretically should have been completely excluded. The alternative model, MIM, is based on the idea that

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aggregates contain water that is immobile and can remain there for some time during the drying cycle and that inter-aggregate space contains immobile water that drains quickly. MIM predicts that habitable pore space for microfauna is available within aggregates even in very dry soils (Glasbey et al. 1991, Griffiths et al. 1995).

In order to understand burrow soil as habitat for nematodes, we compared the pore structure of aggregates from bulk and burrow soils. Our hypothesis was that aggregates from earthworm burrow walls have greater pore volumes in the range of nematode body diameters thus allowing greater nematode abundance observed in these structures. We sampled burrow and bulk soil from mesocosm experiments (Savin et al., 2004) and measured pore size distribution with mercury intrusion porosimetry (MIP, Ritter and Drake, 1945).

Materials and Methods

Soils were taken from mesocosms constructed as a part of a study described previously (Savin et al., 2004). The mesocosms were 50-cm long and 10-cm in diameter, and filled to a depth of 40 cm with a sandy loam soil to a density of approximately 1.3 g cm^{-3}. Two earthworms were added to each mesocosm. 9 g dry-weight of shredded and pre-incubated corn litter was added to the mesocosms. The mesocosms were incubated at 18 – 20° C for a maximum of 16 weeks. Three mesocosms were destructively sampled after 0, 1, 3, 5, 7, 10, 12 and 16 weeks incubation. As part of the sampling, aggregates for MIP analysis were also collected from the burrow and bulk soil after 0, 1, 3, 5, and 16 weeks of incubation. We defined burrow soils as soils within a 5 mm distance from the burrow wall. Non-burrow soil was sampled at least 5 cm from any burrow wall.

Nematodes were extracted from 20 g of each soil type using modified Baerman Funnels as described in Savin et al. (2004). Body diameters of 249 nematodes were measured using image analysis (AxioVision,Carl Zeiss, Thornewood, NY) as the ratio of projected area and nematode length (Browning et al., 2004).

Pore size distributions of soil aggregates, desiccated overnight, were determined with mercury intrusion porosimetry (e.g., Lawrence, 1978; Pachepsky et al., 1995). Three to four determinations were made for each sampling date and soil type using 1 to 1.2 g of desiccated aggregates from each soil type. They were then placed in a powder penetrometer. The penetrometers, sample holders with long narrow stems attached to a bulb, were mounted in the low pressure port of an AutoPore 9420 (Micromeritics, Norcross, GA) Mercury Intrusion Porosimeter. Prior to analysis, the samples were outgassed in the penetrometers to a stable pressure of 3 Pa. Then the penetrometers were flooded with mercury while maintaining 3 Pa pressure. Subsequently, pore volumes were measured as the volume of mercury consumed by the sample, and thus lost from the penetrometer stem, at increasing pressure, each pressure equivalent to a pore diameter. Determinations were made over a diameter range of 100 µm to 0.0049 µm. Specific intrusion volumes were calculated for each pressure interval as the mercury volume imbibed divided by the desiccated sample weight, corrected for moisture measured gravimetrically from an aliquot of the desiccated sample. Samples were allowed to equilibrate for 5 minutes between pressure steps. Hydraulic pore diameter, D, and intrusion pressure, P, are related via a capillary equation (Washburn, 1921):

$$D = -\frac{4\gamma \cos(\alpha)}{P} \qquad [1]$$

where α the contact angle of mercury on mineral soil surfaces and γ the surface tension of mercury. The equation was originally designed for cylindrical pores. However, when defining the pore radius as the ratio of cross-sectional area to perimeter (hydraulic radius), the equation becomes applicable to other pore geometries.

Although the mercury system in the AutoPore 9420 was sealed, precautions were taken to protect workers to mercury exposure during the analysis and the cleaning of sampling equipment by using gloves, protective clothing, positive pressure environment and eye protection.

It is important to note that the pore volume measured with MIP is congruent with the concept of accessible pore space (Postma and VanVeen, 1990). MIP forces mercury, a non-wetting fluid, step-wise into an aggregate at known pressures (Fig. 1). The pore neck size through which intrusion occurs is controlled by the applied

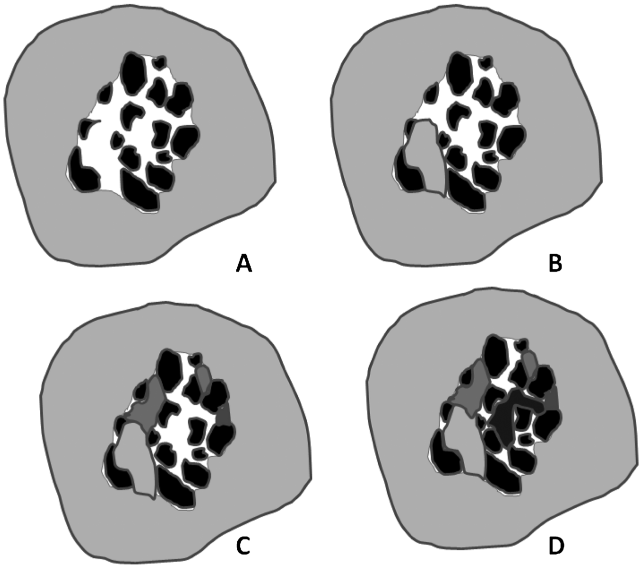

Fig. 1. Intrusion of mercury into an aggregate. Soil solids are shown in black. Mercury is shown in grey. A. Mercury engulfs soil aggregate prior to applying pressure. B. increased pressure results in surface intrusion through a large pore; mercury intrudes until it reaches a pore smaller than the current intrusion pores. C. As pressure increases further, mercury intrudes further into the sample as well as filling small surface pores. D. Almost the entire pore space s filled at even higher pressures, but some pore space remains inaccessible to mercury.

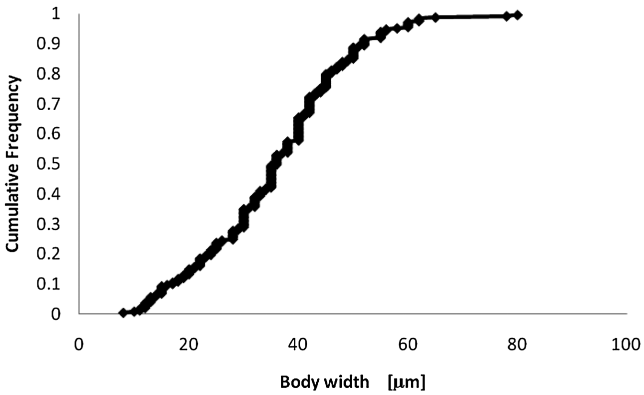

FIG. 2. Cumulative distribution function of nematode body diameters (n = 249).

pressure, whereas the volume depends on the pore space that can be accessed through that pore neck.

Initial intrusion occurs through large surface pores (Fig. 1B). Mercury proceeds into the aggregate until it encounters a pore neck smaller than the hydraulic diameter of the pores calculated with equation 1. As pressure is increased mercury intrudes further into the aggregate from pore spaces already filled and through the aggregate surface (Fig. 1 B – D). In this way, the pore size distribution determined by MIP gives the volume accessible to soil organisms with body diameters equal to or less than the intrusion pore sizes.

ANOVAs were applied to the porosimetry data and pair-wise comparisons carried out where appropriate.

RESULTS

Nematode diameters ranged between 7 and 80 µm (Fig. 2). The median diameter was 38 µm and the interquartile range was 28 to 45 µm. The minimum nematode size was similar to nematodes extracted from intact cores (Gorres et al., 1999) taken from the same soil and field from where the soil for this study originated. However, the maximum and median nematode diameters measured in this study were approximately double of those reported in Gorres et al. (1999).

The average total specific pore volume in bulk and burrow soil did not significantly differ in samples taken on the same sampling day. However, total pore volume increased from the initial value of 0.130 mL/g of soil to 0.177 mL/g of soil after 16 weeks of incubation (Table 1). ANOVA detected differences in total specific pore volume among samples taken over the incubation period ($p < 0.001$). However, only those taken after 16 weeks had significantly greater total pore volumes than those taken during week 0, 1, 3, 5 weeks incubation ($p< 0.03$). All other pair-wise comparisons did not yield significantly different total specific pore volumes.

Pore size changes in specific pore diameter ranges occurred very quickly. After one week of incubation, pore size distributions of both the burrow and the bulk soil differed substantially from the initial distribution (Fig. 3). This might have been a result of packing nd settling of the soil columns. For both soil types taken after 1 week, the pore volume in the 10 to 100 µm range was less, and the volume of pores with pore neck diameters between 5 and 10 µm was greater than in the initial soil. While the change in aggregate pore structure that occurred from the initial to the 1-week aggregates was very pronounced, pore volumes of burrow and bulk soil began to diverge more subtly for pores between 20 and 30 µm (Table 1).

Over the incubation period, soil aggregate pore volume of burrow and bulk soils differed significantly for at least 2 pore intervals within the range of nematode body diameters on each sampling date (Table 2). For example, soil aggregates harvested from the mesocosms after 16 weeks of incubation had significantly different pore volumes in the range from 6 to 31 µm. (Table 2 and Fig. 4).

DISCUSSION

Pore volumes intruded through pore necks with hydraulic diameters similar to nematode body diameters were greater in aggregates from earthworm burrow walls than in aggregates from bulk soil. This is in contrast to results for the pore space of earthworm structures collected from a silt loam (Gorres et al., 2001), where bulk soil had greater pore size volume in the range of 3 – 100 µm than casts or burrows. The discrepancies may be due to the texture differences between the soils used in the two studies (silt versus sandy loam) or the fact that, in the previous study, soils were collected fresh from a fallow field with few detrital resources whereas in this study aggregates were formed in a mesocosm experiment with ample detritus added. Nematode abundance was not measured for the silt loam and a direct comparison between nematode sizes and specific pore size ranges was thus not reported (Gorres et al., 2001).

TABLE 1. Total specific pore volume of bulk and burrow aggreagers at different incubation times.

Time [weeks]	0	1		3		5		16	
		Burrow	Bulk	Burrow	Bulk	Burrow	bulk	Burrow	Bulk
	mL of mercury/g ofsoil								
Average	0.130	0.124	0.131	0.139	0.130	0.136	0.130	0.176	0.177
Standard Deviation	0.004	0.008	0.001	0.014	0.020	0.012	0.014	0.010	0.020

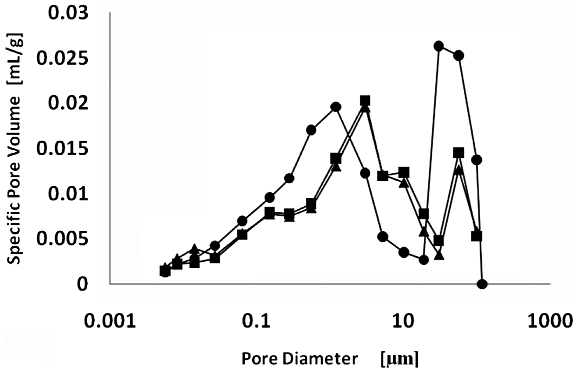

Fig. 3. Comparison of pore size distributions of aggregates taken from initial (circles), burrow (triangles) and bulk (squares) soil after incubating mesocosms for one week.

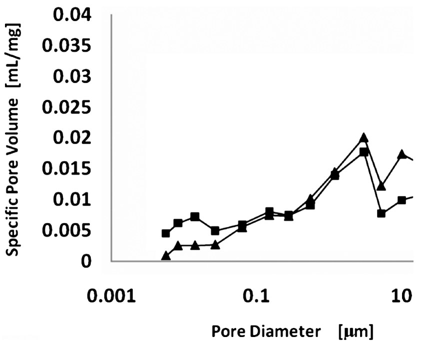

Fig. 4. Comparison of pore size distribution of burrow (triangles) and bulk (squares) soil after 16 weeks incubation.

The mechanisms by which habitable and accessible pore space develop in the burrows are uncertain. One explanation may be that anecic earthworms translocate detritus into their burrows affecting the distribution of organic matter in aggregates (Marinisssen and Hillenaar, 1997). Earthworms weld detritus to the burrow walls by covering it with excrements (Jongmans et al., 2001). Subsequent decomposition of welded organic detrital matter might create new pore spaces in the burrow wall. Alternate mechanisms may involve biogenic micro-aggregates which form in burrow and cast soils and contain small organic matter particles (approximately 50 μm in diameters) (Jongmans et al., 2001). In pot experiments, earthworms have been shown to form calcite spheroids within 3 weeks of worm additions (Ponomareva, 1948, as cited by Jongmans et al., 2001) and micro-aggregates were found to form within 12 days (Bossuyt et al., 2006). The rate of formation of aggregates in earthworm burrows is thus similar to the rate at which pore space developed in this experiment.

Hierarchical aggregation processes where smaller aggregates make up larger ones (Tisdale and Oades 1982; Curry; 1966) may result in distinctive pore space partitioning. Pore spaces within aggregates are generally smaller than the surrounding inter-aggregate pore space at each level of aggregation (Curry, 1966). When biogenic micro-aggregates form in macro-aggregates, void space within the macro-aggregate may have pore necks similar to nematode diameters at some level of the aggregation hierarchy.

Table 2. Probability, p(t), of rejecting H0 (bulk and burrow specific soil pore volumes are not different for a pore diameter interval and sampling week) erroneously for pore size intervals.

Pore Diameter [μm]	Probability p(t), (burrow > bulk)			
	Week 1	Week 3	Week 5	Week 16
56 - 99	0.37	0.03	0.10	0.01
30 - 56	0.12	0.01	0.09	0.07
21 - 30	0.01	0.02	0.05	0.31
12 - 21	0.04	0.28	0.01	0.02
6.4 - 12	0.29	0.06	0.03	0.01
3.5 - 6.4	0.46	0.03	0.49	0.02
1.5 - 3.5	0.07	0.50	0.41	0.07
1.0 - 1.5	0.21	0.38	0.14	0.29

Translocated detrital plant material with internal pore structure may also provide nematode habitat. The lumens of some plant cells have pore diameters in the range of nematode diameters. Wood chips used to stabilize paths across dunes had pore diameters similar to body diameters of nematodes and had been colonized by nematodes (Goralczyk, 2002). However, Amador et al. (2005) reported that the corn litter, applied in the mesocosm used in this study, was quickly decomposed and that only a small amount of litter was found in the burrows at any time during the incubation so that it was unlikely that intact lumens contributed to the pore structure of these aggregates.

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