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Developing a soil quality test with 2D terraria and *Aporrectodea caliginosa*

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Abstract

Burrowing and casting of Aporrectodea caliginosa in 2D terraria is used as an indicator of soil quality.
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Earthworms as indicators of soil quality

It is generally accepted that an abundant earthworm population is characterizing a fertile soil (MAKESCHIN 1997). Earthworms are sensitive to the soil pH and to toxic chemicals in the soil. They are also influenced by the quantity and quality of soil organic matter, by the microbial biomass, by the proliferation and turnover of plant roots and they have a pronounced preference for soil areas with high humidity (NUUTINEN & BUTT 2004).

Earthworm test for soil quality

Aim of the project presented here is the development of an earthworm test for soil quality that is broadly applicable even without laboratory background and helps in decision making in the following fields:

- Testing of soil amendments (fertilizers, pesticides, composts)
- Testing of biological soil quality for land use planning
- Evaluation of forest soils after liming
- Evaluation of soils after clean-up and remediation
- Interpretation of field monitoring data: were earthworm population changes caused by climate or by soil degradation?

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2D terraria as a research tool

2D terraria, “Evans boxes” or “earthworm cuvettes” were invented by EVANS (1947) and have been a standard tool on the investigation of earthworm biology (burrowing behaviour, feeding behaviour, cast production, inter- and intraspecific interactions). They have also been used to asses soil substrate quality (EMMERLING & PAUSCH 2001, FISCHER & EMMERLING 2008, LEIßNER et al. 2008). Various cuvette sizes are reported in the literature. Also the procedures for filling the cuvette with soil and adjusting the water content have been different. For the desired test the set up has to be standardized and simplified.

Material and methods

Cuvettes are 29 cm x 21 cm with wooden frames and a distance between glass plates varying from 5 to 15 mm. A metal dividing sheet was inserted in the middle and the left and the right half of the cuvette were filled with different soil substrates to a height of 15 cm approximately. Filling was done in 3 cm layers which were compacted to 1.4 g cm^{-3} with a punch.

In the first series of experiments with cuvette width 15 and 7 mm the soils were adjusted to a water content of 70 % water holding capacity (WHC) and sieved through 5 mm mesh prior to filling into the cuvette.

In the second series of experiments with cuvette width 5 mm air dry soils were sieved through 2 mm mesh and filled into the cuvette. Water was added by placing the cuvette into a water bath and letting drain afterwards or by capillary rise with a nylon wick connecting the soil to a water reservoir. The water content in these experiments corresponds to the WHC.

After equilibration of the water content earthworms (*A. caliginosa* from a laboratory culture) are placed on the soil surface, the cuvette is closed with adhesive tape and put into the dark at 15°C. Every experiment was done in 4 replicates.

After 3, 5, and 7 days visible earthworm traces from both sides of the cuvette were recorded by hand on transparencies using different colors for burrows and casts. The transparencies were scanned (resolution 75 dpi) and the files were processed by a

computer programme (Excel visual basic). The programme fills the outlined spaces, calculates trace areas in mm² and exports the results into a database (cf. fig. 3). An uneven distribution of traces between the two halves of the cuvette was evaluated with a paired t-Test.
For comparison earthworm avoidance tests with *Eisenia fetida* were made according to DIN ISO 17512-1. Ten adult *Eisenia fetida* from a laboratory breed were put in boxes that were filled with two different soils. After 48 hours the number of worms in each soil was recorded.

Results

Time for development of traces and effect of cuvette width

Figure 1 shows the development of burrow area and of cast area after 3, 5, and 7 days in cuvettes filled with two soils from tree nurseries. The soil on the left side ("Entec soil") had been fertilized with Entec® and is lower in pH and in organic carbon content compared to the reference soil in the right half of the cuvette. While the area of casts is clearly increasing from day 3 to day 7 the burrow area remains rather invariant during this time. In narrow cuvettes (width 0.7 cm) the burrow area is slightly larger than in those with 1.5 cm width. The worm cast area seems not to be influenced by the cuvette width. A preference of the worms for the reference soil is significant already at day 3 and remains through the other reading times. It can be concluded that an incubation time of 3 days is sufficient for the development of a typical burrow area and for the detection of preference/ avoidance in the comparison of the two soil substrates. The width of the cuvette was only of minor influence.

Influence of stocking density and different outcome for burrows and casts

Figure 2 shows the comparison of two arable soils in the cuvette, a luvisol chernozem and a floodplain cambisol. The soils are differing in color. Therefore it was possible to detect if soil was taken up by worms in one half and defecated in the other half of the cuvette. It can be seen that doubling of the stocking density to 8 worms resulted in a decrease of the burrow area and in an increase of the cast area. Burrows are probably refilled with casts more frequently at the higher stocking density. At 4 worms stocking density there was a significantly larger burrow area in the luvisol chernozem while the cast area was significantly larger in the floodplain cambisol. In the right half of the cuvette (floodplain cambisol) 35 % of the cast area had the dark color of the chernozem soil while in the left half only 11 % of the cast area was originating from the cambisol. It can be hypothesized that the worms fed more in the chernozem but rested more in the cambisol.
The influence of stocking density was also investigated in a sandy cambisol (Refesol A01; http://www.refesol.de/) wetted to 100 % WHC (tab. 1).

Tab. 1 Influence of stocking density on earthworm-trace area in dip-watered cuvettes with sandy cambisol (Refesol A01) after 3 days (soil too wet for distinction of casts in the soil).

Worms per 100 cm^{-3}	Test setup	Burrow area /cm² per 400 cm²	Surface cast area /cm² per 400 cm²
0.5	29 x 15 x 0.5 cm³ 1 worm	18.9 (6.3)	0.8 (1.4)
1	21 x 10 x 0.5 cm³ 1 worm	26.9 (8.2)	4.6 (2.8)
2	29 x 15 x 0.5 cm³ 4 worms	42.9 (6.1)	4.6 (2.6)
4	21 x 10 x 0.5 cm³ 4 worms	39.5 (9.2)	25.6 (6.1)

There was a linear increase of burrow area per 400 cm² from 19 to 43 cm² with increasing stocking density to 2 worms per 100 cm³. The increase of stocking density from 2 to 4 worms per 100 cm³ resulted in a slightly smaller burrow area and in a drastic increase of surface casts.

Effect of soil water content

Earthworms have a high preference for humidity. Therefore the outcome of a soil comparison test is strongly influenced by the water content adjusted in the soil. In an experiment (results not shown) the soil preference of the worms could be reversed by changing the water content in one half of the cuvette from 70 % to 87 % of WHC. To overcome this problem the cuvette was watered until an equilibrium between capil-

lary uptake and drain off was reached. Figure 3 shows the water contents at six points in a cuvette that was watered for three days with a nylon wick introduced at the right side. The water content was around 100 % WHC with a slightly lower value in the upper left corner of the cuvette. The distribution of worm traces does not indicate any avoidance of wet areas but rather leaves the question if already a water content of 89 % WHC is below the preferred optimum.

Comparison of *A.caliginosa* cuvette test with *Eisenia fetida* avoidance test

Tab. 2 Distribution of *Eisenia fetida* worms and of *A. caliginosa* burrows and casts in various soil pairs. Bold underlined numbers indicate significant preference/avoidance (p<0.5). Lch = luvisol chernozem and Fca = floodplain cambisol from fig. 2. Ow, Wb = apple orchard cambisol and rendzic arable soil, Ent+, Entref = soils from fig. 1. Mn+, Mnref = Mn excess soil and reference. Ca-, Caref = Ca-deficient soil and reference. F-lim, F-ash acid forest cambisol limed and treated with lignite-ash.

Soil A	Soil B	ISO avoid. *E.fetida* in B	Cuvette *A. caliginosa* Burrow in B	Cuvette *A. caliginosa* Cast in B
Lch Ltu pH 7.6 C 1.6%	**Fca** Ls4 pH 5.7 C 1.7%	58%	**<u>39%</u>**	**<u>72%</u>**
Ow Sl pH 6.2 C 2.4%	**Wb** Ls pH 6.3 C 1.6%	**<u>11%</u>**	48%	50%
Lch wc 24%	**Lch** wc 34%	46%	52%	**<u>16%</u>**
Lch wc 24%	**Lch** wc 29%	65%	61%	**<u>64%</u>**
Mn+ Sl pH 4.6 C 3.2%	**Mnref** Sl pH 5.3 C 4.7%	34%	**78%**	**<u>80%</u>**
Ent+ Sl pH 4.1 C 2.4 %	**Entref** Sl pH 6.0 C 4.6%	54%	**96%**	**97%**
Ca- Sl2 pH 5.5 C 1.6%	**Caref** Sl2 pH 4.9 C 4.7%	72%	54%	44%
F-lim pH 4.4 C 11.9%	**F-ash** pH 4.0 C 8.5%	25%	**<u>12%</u>**	28%

For eight pairs of soils the cuvette test with *A. caliginosa* was compared to the *Eisenia fetida* avoidance test according to DIN ISO 17512-1. The results listed in tab. 2 show clearly that the two tests are not interchangeable. The *A. caliginosa* cuvette test also seems to be more sensitive. It indicated a significant difference in burrow area in 4 cases while only one significant preference/avoidance occurred in the *E. fetida* ISO-test.

Conclusions and outlook

- Evan's boxes are suitable for soil testing.
- The ISO Eisenia fetida avoidance test is not equivalent to the A. caliginosa cuvette test.
- Control of humidity is most important and should be adjusted to around 100% water holding capacity.
- Burrows and casts should be recorded separately. The trace area can be measured easily and is a sufficient parameter for the quantification of soil use by the worms. At very high water content the visibility of casts may be reduced.
- An incubation time of 3 days is sufficient for the development of trace area and for the detection of preference behaviour.
- At stocking densities above 2 worms per 100 cm^3 the linear relationship between worm number and burrow area is lost.

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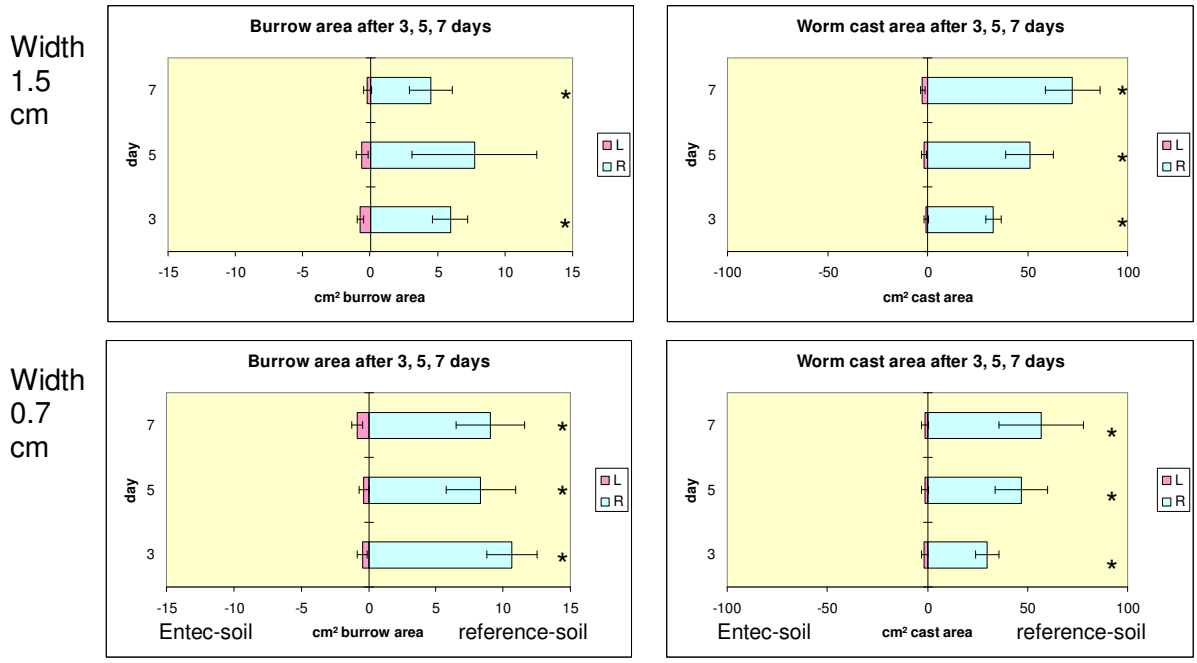

Fig. 1 Area of burrows and casts in cuvettes with "Entec soil" combined with reference soil after 3, 5, 7 days and with two distances between glass plates. Stocking density 6 adult *A.caliginosa*. ["Entec soil": loamy sand, pH 4.12, C_{org} 2.4 %,C/N 18, field capacity at pF 1.8 20 %; reference soi: loamy sand, pH 5.3, C_{org} 4.7 %, C/N 19, field capacity 29 %] * = significant difference between left and right half of cuvette (paired t_test, p < 0.05)

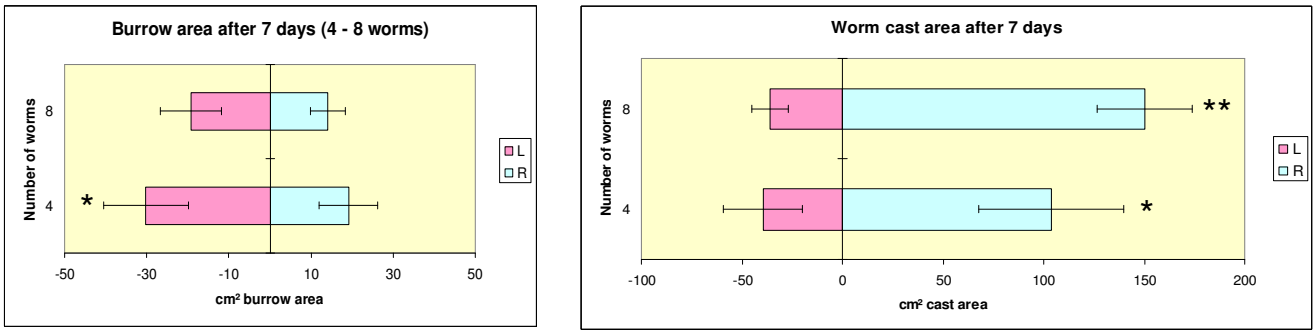

Fig. 2 Burrow area and cast area in cuvettes (width 1.5 cm) with 4 and 8 worms stocking density [left half: Luvisol Chernozem Ltu, pH 7.6, C_{org} 1.6 %, C/N 12, R_{bas} 3.5 µg CO_2 g^{-1} h^{-1}; right half: Floodplain-Cambisol Ls4, pH 5.7, C_{org} 1.7 %, C/N 16, R_{bas} 1.8 µg CO_2 g*-1* h*-1*] * = significant difference between left and right half of cuvette (paired t_test, p < 0.05)

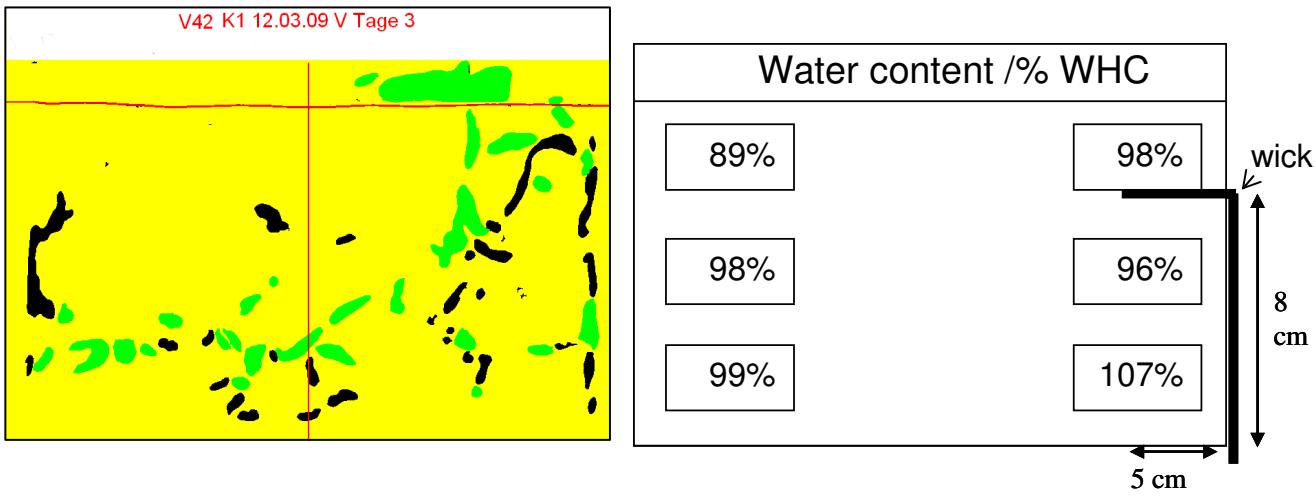

Fig. 3 Traces of 4 adult *A.caliginosa* in a cuvette (width 0.5 cm) filled with dry Luvisol-Chernozem soil and watered with a wick for 3 days. Image after digital processing (green = casts, black = burrows). Right: water content (% of water holding capacity) at 6 points at the end of the experiment.