ELSEVIER

Soil Biology & Biochemistry 39 (2007) 700–703

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Short communication

Motility and migration rate of protozoa in soil columns

S.M. Adl*

Department of Biology, Dalhousie University, 1355 Oxford St., Halifax, Nova Scotia, B3H 4J1, Canada

Received 6 July 2006; received in revised form 24 August 2006; accepted 2 September 2006
Available online 13 October 2006

Abstract

Velocity and distances travelled each day by protists in the soil was previously unknown. Soil columns were designed to monitor the distribution of cells along the length of each column as a way to measure migration potential. Motility was affected by pore space, water flow and gravity. The results show that the soil microhabitats that can be explored and exploited by flagellate, amoeboid or ciliated species is in the order of centimetres per day in sandy and sandy loam-textured soil matrices. This value is useful to understand the soil volume that can be explored for food and protist dispersal potential without lateral water flow.

Keywords: Amoebae; Ciliates; Colonisation rate; Flagellates; Immigration; Soil biota

The velocity of locomotion of protists in the soil is unknown. I constructed soil columns to determine whether it was possible to address the motility of soil protists based on the ideas derived from chromatography (Jönsson, 1987). The soil matrix mimics a porous chromatography column, or gel, packed to provide a variety of pore sizes that exclude some cells but permit passage of others. Packing of the soil columns (bulk density and particle density) therefore affects porosity and acts to retard the directional locomotion of some species relative to others. In more tightly packed columns, smaller pore spaces exclude cells that are too large to pass through, and retard the mobility of larger or inflexible cells. The relationship among compaction, pore-size distribution and tortuosity are not simple (Sparks, 1999) but would affect the migration velocity of cells. The soil columns provided a stationary phase (the soil matrix) covered in water, which is pulled by gravity (the fluid phase). The soil column construction focused on the soil textures that provided the fastest migration rates. The results were used to obtain estimates of the range of locomotion velocities in soils of varying texture and porosity.

The soil for this study was obtained from the Horseshoe Bend Long-term Agricultural Research Field site at the University of Georgia (Athens, GA). Soil was air dried and sieved through a succession of mesh sizes (1 mm, 500, 250, 180, 53 μm). Sand free of organic matter (Fisher Scientific) was sieved through the same mesh sizes. Each soil and sand-size fraction in Table 1 consisted of 2:1 ratio of sieved soil and sieved sand of the same size fraction to provide a range of texture and porosity. We established by trial and error that the sandy end of soil texture provided faster locomotion rates. The mixed soil and sand fractions were autoclaved 20 min and stored under sterile conditions at room temperature. Soil columns were constructed using clear Perspex cylinders with holes of 5 mm diameter drilled at 2 cm intervals along one side of each cylinder (Fig. 1). The cylinders were sterilised in 10% bleach solution for 2 h, rinsed twice in sterile dH_2O, submerged in 95% ethanol for 10 min, then placed on a rack in a sterile flow-hood until dry. The side with holes was covered with a strip of masking tape, and the cylinders were capped at each end with sterile aluminium foil held in place with elastic bands for storage. The columns and the soil were handled in a sterile-air flow-hood using sterile techniques throughout the column assembly and experiments.

Column percentage pore space was calculated from the bulk density and particle density as described by Robertson et al. (1999). The powdered organic wheat grass (Pines Int., Kansas) was evenly mixed into the soil as a source of nutrients (Sonneborn, 1970). The sterile medium consisted

*Tel.: +1 902 494 2753; fax: +1 902 494 3736.
E-mail address: sadl@dal.ca.

0038-0717/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2006.09.008

Table 1
Composition of soil in columns assembled from sieved fractions

Component	Column A (%)	Column B (%)	Column C (%)
Sand and soil fraction			
1–0.5 mm	29	14	21
500–250 μm	71	42	0
250–180 μm	0	44	25
180–53 μm	0	0	18
<53 μm	0	0	36
Wheat grass (g)	0.1	0.1	0.1
Medium (ml)	21	21	21
Moisture (%)	30	30	30
Bulk density ($g\,ml^{-1}$)	1.10	1.05	1.02
Particle density ($g\,ml^{-1}$)	1.25	1.25	1.25
Pore space (%)	12	16	18.4
Texture	Sand	Sand	Sandy loam

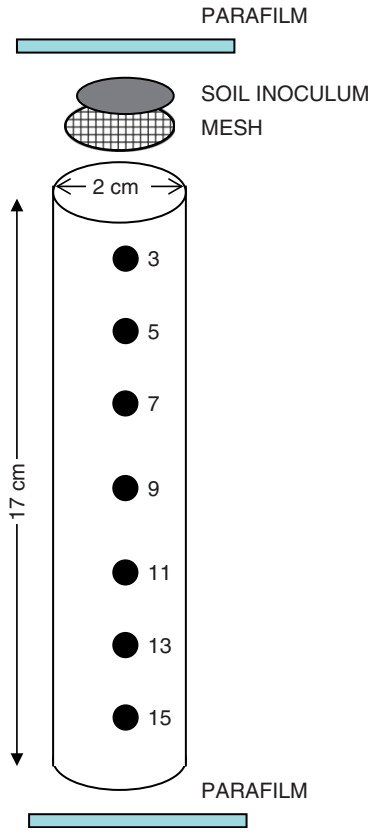

Fig. 1. Soil column design.

of 25 mM sodium citrate, 25 mM ammonium nitrate and 25 mM sodium bi-phosphate in dH_2O, with a final pH of 6.3. The moistened columns were allowed to stand one day to equilibrate and for bacteria to grow. The source of the bacteria was from those surviving in the soil. About 50 g fresh field soil for the inoculum was gently mixed with a spatula before weighing 2 g subsamples. A pulse of protozoa was obtained by placing the inoculum on a 45 μm Nitex mesh (Fisher Scientific) on top of each column for one day. Each inoculated column was covered with Parafilm to prevent drying and incubated at 25 °C in the dark.

In a preliminary trial, two columns each of type A, B and C were established and inoculated with fresh soil using a thin spatula midway at the 9 cm hole, and one un-inoculated control column of each A, B and C. This showed that over several days protists would grow and move vertically in both directions from the site of inoculation (data not shown). This was important to verify that gravity was not having an overwhelming effect on the column by pulling cells down through the soil.

The experiment consisted of one column A, four columns B and one column C. One column B was not inoculated to serve as a control. The control columns remained free of invertebrates and protists throughout the experiments but contained bacterial growth. The remaining B columns were inoculated and incubated vertically (day 0). On day 1, one column B was placed horizontally (B–H), and another column B was placed under dripping sterile (autoclaved) water at $0.25\,ml\,h^{-1}$ (B–D). The entire experiment was independently replicated three times.

At daily intervals, a subsample of soil from each side hole was removed under sterile conditions with a spatula. The soil was weighed and placed into 300 μl of medium already pipetted into 24 well plates (Nunc®, Fisher Scientific). The plates were examined within two hours using an inverted microscope (Nikon) at × 100 and × 200 magnification with phase contrast. The number of protists in each well was counted and categorised into four motility groups as <12 μm flagellates, >12 μm flagellates, amoebae and ciliates. The abundances, calculated as the number of cells g^{-1} soil, were used to calculate the mean and standard error at each point along the column and graphed. The subsample dry weight was calculated from the known moisture content of each column. The standard error of the mean ($n = 3$) was 11% or less for all calculated values. This indicates very good reproducibility of the results. From the graphs we obtained the mean, median and mode of distributions for each column (Table 2). Estimated velocities were compared by two-tailed *t*-test, and mean distance travelled by repeated measures ANOVA with three levels of texture (columns A, B and C) or three levels of column B (columns B, B–H, B–D) as the factor. All statistical procedures followed Zar (1981).

Bacteria abundances were estimated on a haemocytometer grid from soil subsamples stained with DAPI (4′,6-diamidino-2-phenylindole, Sigma) (Robertson et al., 1999). Bacteria abundances were $10^7\,g^{-1}$ by the end of day 1, throughout each column. The bacteria abundances increased slightly during the course of the experiment but remained below $10^8\,g^{-1}$ in the ungrazed portion of columns. The bacteria abundance in the grazed portion of the columns was reduced below $10^6\,g^{-1}$.

Protists were detected at the top of the columns during day 2, throughout the columns at day 4 and the head of the migrating wave reached the end of most columns by day 5. Two-tailed paired sample *t*-tests comparing column B to