

# Quantitative image analysis of earthworm-mediated soil displacement

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Received: 23 March 2009 / Revised: 11 June 2009 / Accepted: 22 June 2009 / Published online: 5 August 2009  
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**Abstract** The way soil is disrupted and deformed by earthworm movement is hard to quantify non-destructively. Two anecic earthworm species, *Aporrectodea longa* (Ude) and *Lumbricus terrestris* L., were compared in their effect on the soil around them as they burrow. Image analysis (particle image velocimetry, PIV) was used successfully to quantify the distance and direction of soil particle displacement by earthworm locomotion giving a unique insight into their effect on the soil structure development. The data collected from both species using PIV show a decline in soil displacement at increasing distance from the earthworm's body. The quantity of soil being displaced was more to the sides of the earthworms (radially) than in front of the prostomium (axially). Also, *L. terrestris* displaced more soil than *A. longa* both axially and radially. The findings from this study suggest that PIV image analysis is a viable tool for examining soil displacement by earthworms and the method used has the potential to be applied to other soil organisms.

**Keywords** Earthworms · Particle image velocimetry · Burrowing · Soil displacement

## Introduction

Earthworms modify their environment by changing the physical and chemical properties of soil, through creating

burrows and excreting casts (Brown 1995). Burrows created by earthworms, as with all macro-pores, disrupt the soil matrix and can provide pathways for plant roots to grow deep into the soil. Edwards and Lofty (1978), Ehlers et al. (1983) Hamilton and Dindal (1989) all conclude that earthworm activity enhances root growth through producing pathways of less resistance, improving water infiltration and through soil aeration. The anecic species are known for their turnover of nutrients, as they may draw leaf litter into deeper layers of the soil (Bolton and Phillipson 1976).

Burrowing earthworms move the soil either by ingestion or by displacement. For example, *Lumbricus terrestris* will ingest dense soil but may simply displace less dense material (Buck et al. 2000). Darwin (1881) and Newell (1950) observed that alternating contraction and expansion of the anterior body occurs as the earthworm enters the soil from the surface. The anterior of the earthworm will elongate pushing the prostomium and the first segments into the soil. These segments will expand to push the soil away to each side of the body. The anterior can then be anchored into this soil; the prostomium samples the soil around it, possibly to find the path of least resistance.

Measuring the movement of burrowing organisms and quantifying the effect on the soil around them is difficult. As a result, pressure and compaction of earthworm burrowing has been tested in a number of experiments using differing methods. Soil discs of differing tensile strengths have been used to study the radial pressure exerted by the species *Aporrectodea rosea* (Savigny; McKenzie and Dexter 1988a). More recently, photography and image analysis have been used to study the affect of burrowing in soil over a period of time (Schrader 1993). The impact of burrow systems on soil structure and bio-pore volumes has also been studied using X-ray computed tomography (Langmaack et al. 1997; Jégou et al. 1999;

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Capowiez et al. 2003; Schrader et al. 2007). Force exerted by earthworm burrowing has been studied using artificial burrows that were enlarged by earthworm burrowing and the force was measured using a strain gauge (Quillin et al. 2000). Despite these advances in approaches to observing earthworms in the soil environment, our understanding of soil structure development by burrowing is relatively poor, due to the difficulty in quantifying these processes.

Adrian (2005) described particle image velocimetry (PIV) as the “accurate, quantitative measurement of fluid velocity vectors at a very large number of points simultaneously”. PIV is a measuring technique for the non-invasive study of the velocity of image features or particles. Particle image velocimetry measures the displacement of particles (Grant and Liu 1990) between successive images (White et al. 2003). The technique is widely used to measure the displacement of particles in fluid mechanics and geotechnical testing (White et al. 2001). Improvements in computers have simplified PIV analysis, and digital cameras allow for time-lapse photography to be used more readily. These advances have opened up PIV for use in many areas of engineering and science.

The use of PIV in zoology is relatively recent, but it is an important step in understanding animal movement through air and fluids. Drucker and Lauder (1999) used PIV to study momentum transfer from animal to fluid, studying the movement of fish in water and quantifying the movement. Also, Hendenström et al. (2006) used PIV to study the wake vortices of passerine birds in flight.

Bio-pores that result from earthworm burrowing influence soil function through the turnover of nutrients, filtering of water (Edwards and Shipitalo 1998) and minerals, diffusion and mass flow of gasses and producing access points for roots to reach deeper soil. We aim to quantify the movement of two anecic species (*Aporrectodea longa* and *L. terrestris*) and the soil around them using PIV analysis with Matlab (Mathworks 2000). These two species were used because both species are classified as anecic and may be expected to have similar burrowing habits. We test whether PIV analysis is a viable tool for measuring soil deformation by earthworms, in particular, and perhaps other fauna, in general. We suggest that this method may provide a new way to analyse both the behaviour of soil animals and a new way to quantify their effect on soil structure.

## Materials and methods

### Soil and earthworms

Soil was collected (0–20 cm) from an ungrazed pasture at The Scottish Crop Research Institute, Dundee, Scotland (56°27' N, 3°40' W). The soil is derived from undifferen-

tiated sandstone. Known locally as Balrownie series, it is a Stagnic Cambisol according to the World Reference Base classification, with 660 g kg<sup>-1</sup> sand, 230 g kg<sup>-1</sup> silt and 110 g kg<sup>-1</sup> clay, with a pH (CaCl<sub>2</sub>) of 5.7 and soil organic carbon at 38 g kg<sup>-1</sup>. Soil was sieved to <2 mm and stored at room temperature in a sealed container.

Adult and sub-adult *A. longa* were collected by hand from the top soil (0–20 cm) of the same ungrazed pasture that had been used for soil collection (September 2008). The earthworms were identified by external features using the key of Sims and Gerard (1985). Adult *L. terrestris*, unavailable by collection, were supplied by Worms Direct (Essex, UK) and identification confirmed by the same key. All earthworms were kept in a sealed container with turf at field capacity and refrigerated at 7°C.

### Initial tests

To study earthworm burrowing a viewing box was constructed from 2-mm-thick Perspex (160×160×5 mm). The 5-mm width allowed even the largest of the *L. terrestris* individuals to burrow freely into the soil in the viewing box. The front face of the box could be unscrewed, to allow the soil to be packed and earthworms were encouraged to burrow vertically using two pieces of flexible, transparent plastic centrally placed at the top of the viewing box.

To pack the box, soil was sieved to <250 µm and 128 g mixed with water to a water content of approximately 0.3 g g<sup>-1</sup>. Actual soil water content was determined after testing. The soil and water were mixed to produce a visually even consistency. The transparent side of the viewing box was removed to allow the soil to be packed evenly to a dry bulk density of 0.88 g cm<sup>-3</sup>. Small amounts (~0.5 g) of fine sand were dusted onto the soil at the viewing face to improve image texture for PIV analysis. The front of the viewing box was replaced. The viewing box allowed the earthworms to burrow through the soil environment whilst allowing images to be captured through the transparent sides. The box was emptied and re-packed with fresh soil for each earthworm.

The strength of the soil in the viewing box was determined using a penetrometer (tip Ø=1.96 mm, shaft Ø=1.82 mm, cone angle 15°). The penetrometer, attached to a loading frame, was driven at 4 mm/min into the soil to a depth of 12 mm. Strength was taken as the mean force recorded on a load cell per unit cross-sectional area of the penetrometer cone and expressed as Pa.

Prior to testing earthworm movement using the viewing box, the general pattern of movement was determined for an individual earthworm of each species by capturing time lapse sequences from above whilst the earthworm moved on a flat white surface. Illumination was from above by a desk lamp.

### Main experiment

All imaging was performed using a Nikon D300 camera with a macro-lens (Nikon 60 Micro AF) using a tripod and shutter release cable. Earthworm movement was captured using burst mode (six images per second), and images were 3,000 × 4,000 pixels. A polarising filter decreased unwanted reflection.

Each earthworm was weighed initially, placed onto the surface of the soil in the viewing box and allowed to burrow. After the earthworm had burrowed to a depth of at least 15 mm (on average, earthworms burrowed to 32 mm ± 1.90) into the soil, sequences of images were captured. To capture a full sequence of muscle contraction, at least five images and typically 10 were required.

In total, we analysed six *A. longa* and ten *L. terrestris* each from separate viewing boxes. Comparisons between the boxes and earthworms were analysed by *t* tests in Sigma Stat (version 10).

### PIV analysis of image sequences

The program GeoPIV 8 (White et al. 2001) was used to determine the displacement of patches (user-defined regions of the image) between pairs of sequential images. Patch size measured 50 × 50 pixels (typically 2.05 × 2.05 mm), and the horizontal and vertical displacements ( $d_u$  and  $d_v$ ) were output corresponding to the centre coordinates of each patch ( $u_o$ ,  $v_o$ ).

From  $d_u$  and  $d_v$ , we calculated resultant displacement as  $d_1$  using the data converted into millimetres.

$$d_1 = (d_u^2 + d_v^2)^{1/2}.$$

A standard ruler was used as a reference scale in each image allowing the analysis of soil deformation at known distances from the body of an earthworm.

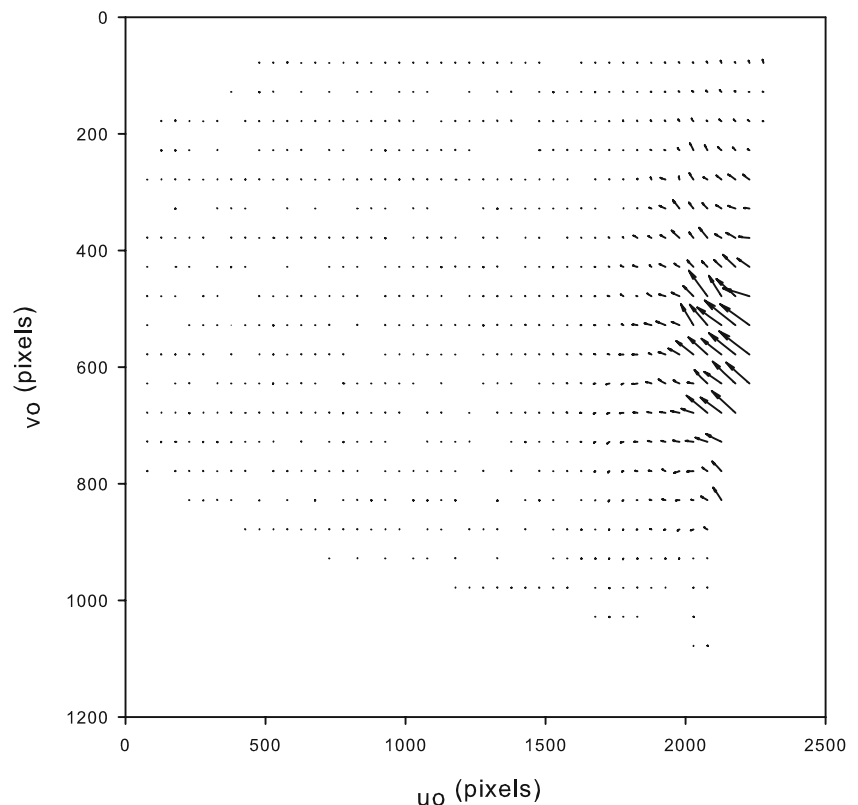
### Results and discussion

#### Initial tests

Preliminary observations and PIV analysis (data not shown) of earthworm movement on a flat surface were consistent with Yapp (1956) who noted that waves of muscle contraction from *Lumbricus* and *Allolobophora* specimens did not extend further than the anterior of the earthworm’s body, whilst the posterior segments dragged passively behind. We therefore concentrated our subsequent analysis on the anterior (prostomium to clitellum) of each earthworm.

We analysed image sequences taken of earthworms in soil that showed a complete movement cycle, as described by Darwin (1881) and Newell (1950). First, the longitudinal muscles contracted extending the earthworm radially and then the circular muscles contracted extending the earth-

**Fig. 1** Quiver plot showing soil displacement vectors to the side of one individual of *L. terrestris*.  $u_o$  and  $v_o$  correspond to direction of displacement,  $u_o$  for x axis and  $v_o$  for y axis



worm axially. We focused our analysis on the longitudinal muscle contraction as being the more dynamic phase and because it was clearly visible for all earthworms of both species.

#### Soil displacement during longitudinal muscle contraction

Particle image velocimetry was run on image sets starting at pre-contraction of muscles and finishing immediately after one contraction was complete. Figure 1, a quiver plot, is an example of the displacement of soil between first and last images of longitudinal contraction. In Fig. 1, only displacement to the left of the image is shown. For each earthworm, the area of the image to be studied was partitioned into axial,

radial left and radial right sections. This partitioning simplified the analysis. Arrows in the image are to scale and show the patch displacement vectors. The data represented by this quiver plot (i.e. the patch displacement vectors) were also saved to a text format that can be imported into Excel spreadsheets or Matlab for further analysis.

There was no significant difference between the soil water content used for the *A. longa* and *L. terrestris* individuals ( $0.320 \pm 0.0041 \text{ g g}^{-1}$ ). Penetrometer resistance was  $15.02 \pm 0.03 \text{ kPa}$  at 12 mm depth. This penetrometer resistance is much less than the mean axial pressures of 72.8 kPa generated by *A. rosea* (McKenzie and Dexter 1988b), indicating that movement of these earthworms will not be inhibited by soil resistance.

**Fig. 2** Soil displacement as a function of **a** radial and **b** axial distance from *A. longa* and *L. terrestris*, data plotted as arithmetic mean  $\pm$  standard error

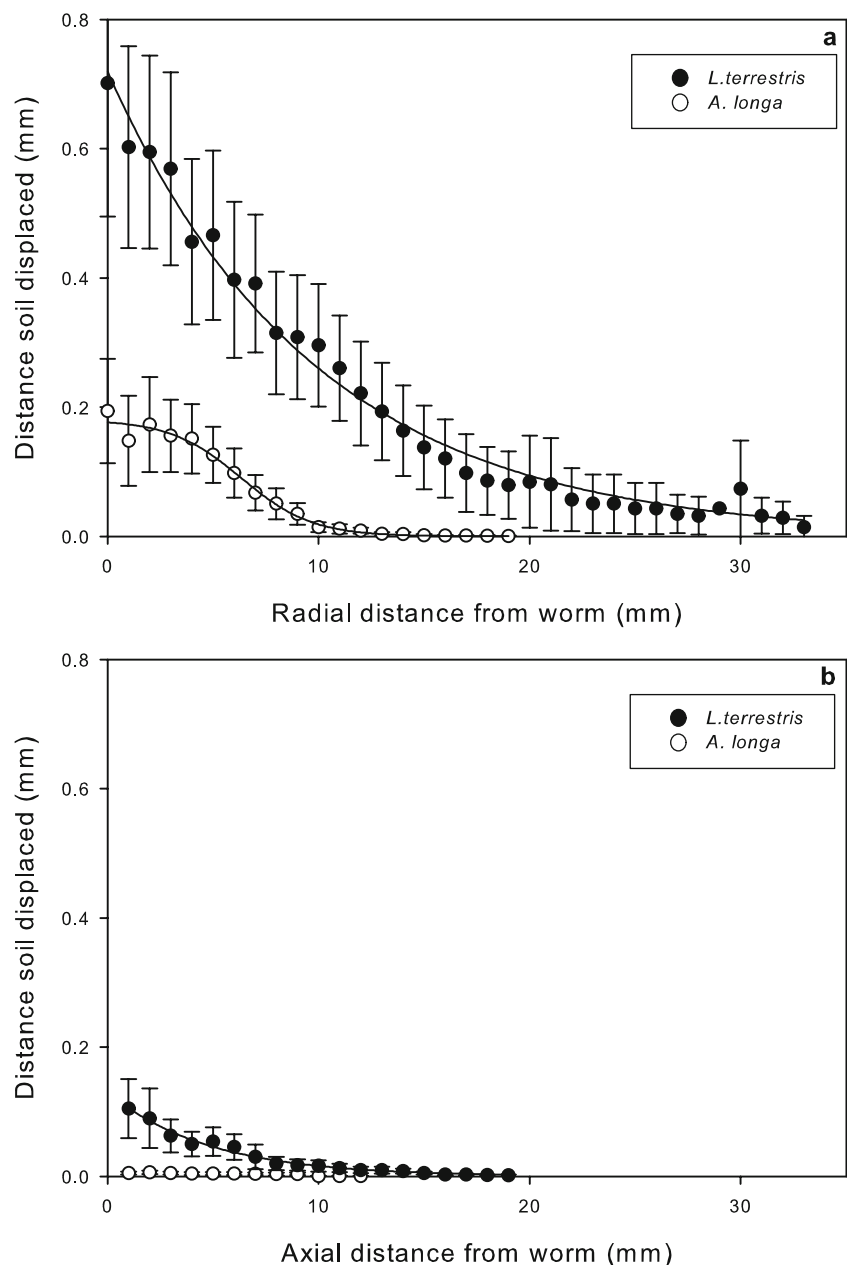
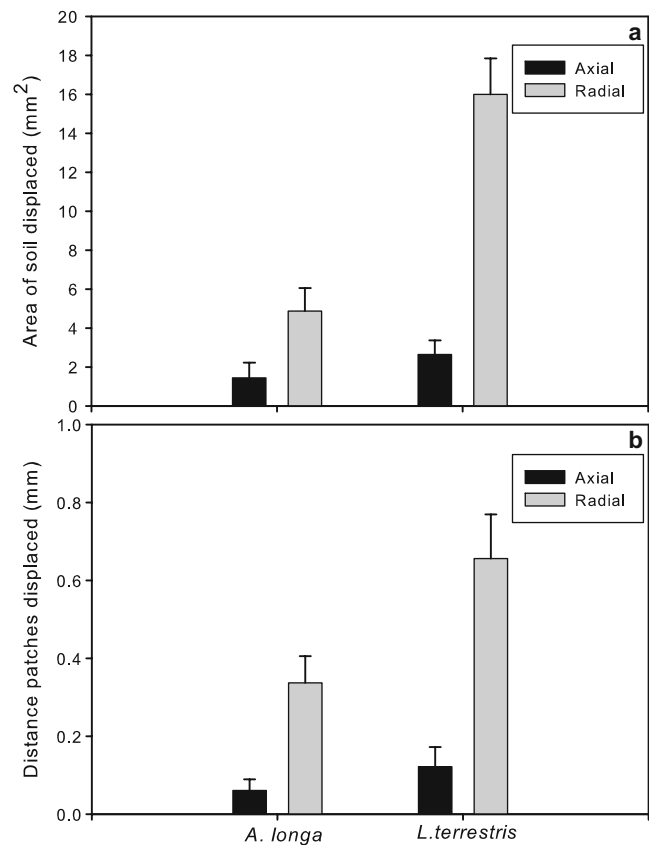


Figure 2a, b compares the mean displacement of soil resulting from the burrowing of the two species, radially and axially during longitudinal muscle contraction. Arithmetic mean soil displacement from *L. terrestris* was significantly greater than for *A. longa* in the radial direction ( $p < 0.001$ ). The axial displacements were not different between species ( $p = 0.800$ ). Mean displacement for *L. terrestris* decreased exponentially as a function of distance from the earthworm body, both axially and radially. The *A. longa* mean displacement followed a sigmoidal relationship with distance. Both species displace more soil closer to their bodies and greater displacement of soil occurs radially than axially, similar to observations by Dexter (1987) on roots, where he found soil density decreased exponentially with distance from the root surface. Greater radial expansion could be due to greater strength in contraction of the longitudinal muscles to produce radial movement during this part of the cycle (McKenzie and Dexter 1988a; Keudel and Schrader 1999; Quillin 2000). Radial compression of the soil will also allow burrow walls to be strengthened by soil compaction. Dorgan et al. (2006) suggest that macro-faunal burrowing may cause stress field cracks, creating areas of less resistance axially and facilitating earthworm burrowing.

Figure 3a summarises the soil displacements in Fig. 2 to compare the mean area (greater than a minimum threshold of background noise) of soil displaced for each species. In Fig. 3b, the distance that the patches are displaced is significantly greater radially in *L. terrestris* than in *A. longa* ( $p = 0.006$ ). However, the axial distance of patch displacement is not significantly different ( $p = 0.804$ ), but it appears that there is a non-significant trend for mean *L. terrestris* to displace patches further than *A. longa* (similar to findings in Fig. 3a for area of soil displaced). The area of soil displaced supported the observations in Fig. 2a, b, comparing the large differences between species and direction of soil displacement. *L. terrestris* individuals ( $5.64 \pm 0.351$  g) were considerably heavier than *A. longa* individuals ( $1.43 \pm 0.142$  g), and *L. terrestris* displaced more soil over greater distances. Figure 4 gives an example of displacement of soil by each species and shows how *A. longa* individuals displace soil over a smaller area (Fig. 4a) compared with *L. terrestris* individuals (Fig. 4b). Segments 6/7/8/9/10 are strongly muscular in *L. terrestris*, whilst *A. longa* are muscular in segments 7/8/9 (Sims and Gerard 1985). These differences, along with the differences in body mass, will affect amount of soil displaced by individuals of each species and provides a physiological basis for our findings.

Keudel and Schrader (1999) found that *A. longa* and *L. terrestris* exert more pressure radially than axially. Our findings of amount of soil displaced (Fig. 2) are consistent with that observation. Keudel and Schrader (1999) in a study conducted out of soil found that *A. longa* exerted a greater maximum pressure than *L. terrestris* axially and radially.



**Fig. 3** Area of soil displaced (a) and the distance these patches displaced (b) adjacent to earthworm (radial) and in front of the earthworm (axial), data plotted as arithmetic mean  $\pm$  standard error ( $n = 6$  for *A. longa*;  $n = 10$  for *L. terrestris*)

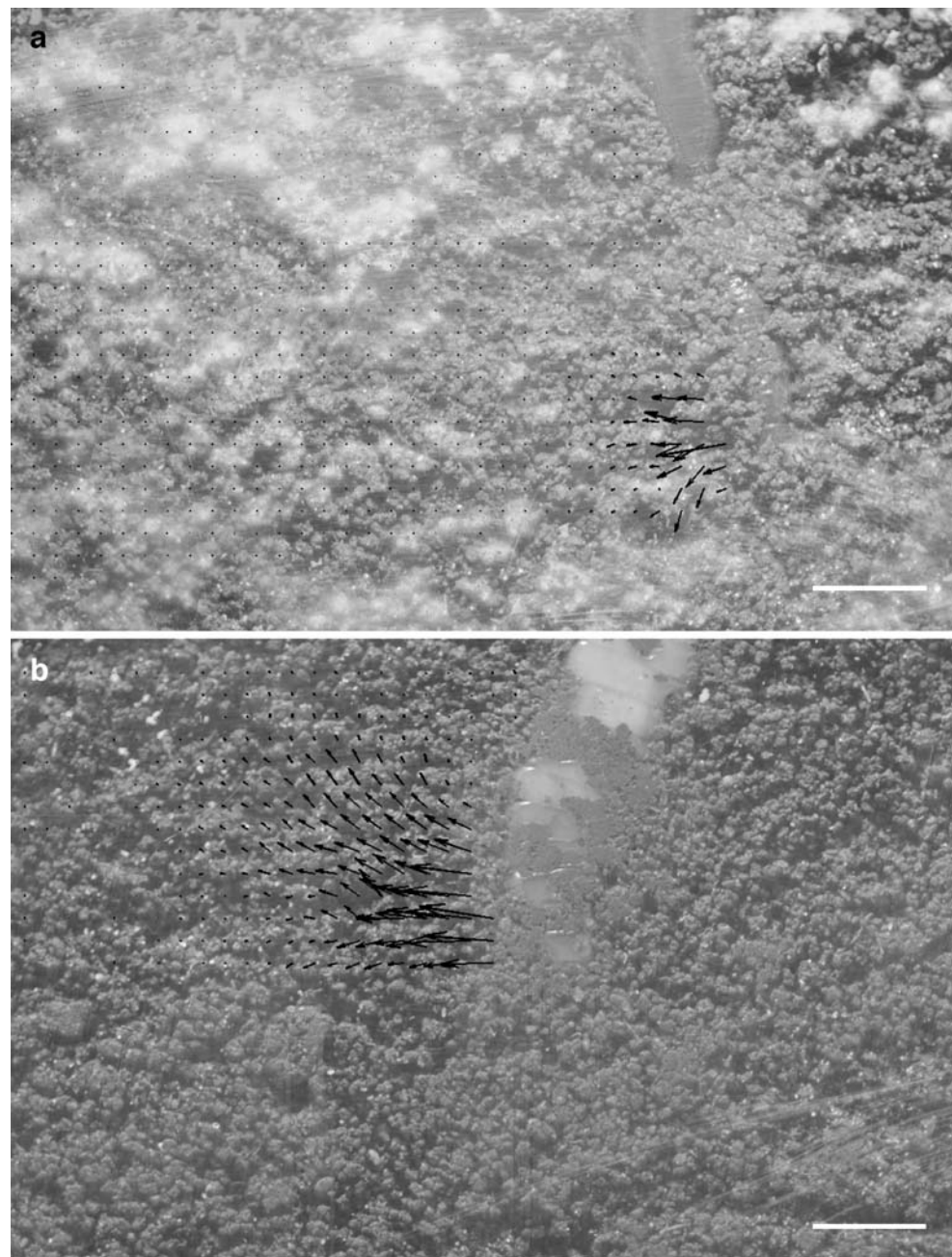
Their findings are not inconsistent with our results of maximum soil displacement. The maximum expansion that an earthworm can achieve is limited by its physiology. To fully understand the interactions between pressures exerted onto soil by earthworms and the amount of soil displaced, we need to both quantify the physiological limitations of the earthworms and better characterise the soil properties.

#### Circular vs longitudinal contraction

Where images were available of sufficient quality, we additionally analysed the circular muscle contraction that results in axial extension of the body. Figure 5 shows a comparison between the effects of circular and longitudinal muscle contractions on soil displacement axially and radially for two *A. longa* individuals and two *L. terrestris* individuals. As expected for all four earthworms examined, the largest displacement occurred radially and at the longitudinal muscle contraction; for three of the comparisons, the radial displacements are at least twice that of axial displacements.

Although we expected that when the circular muscle contracted there would be a greater displacement of soil axially, this was not the case (Fig. 5). The earthworms were

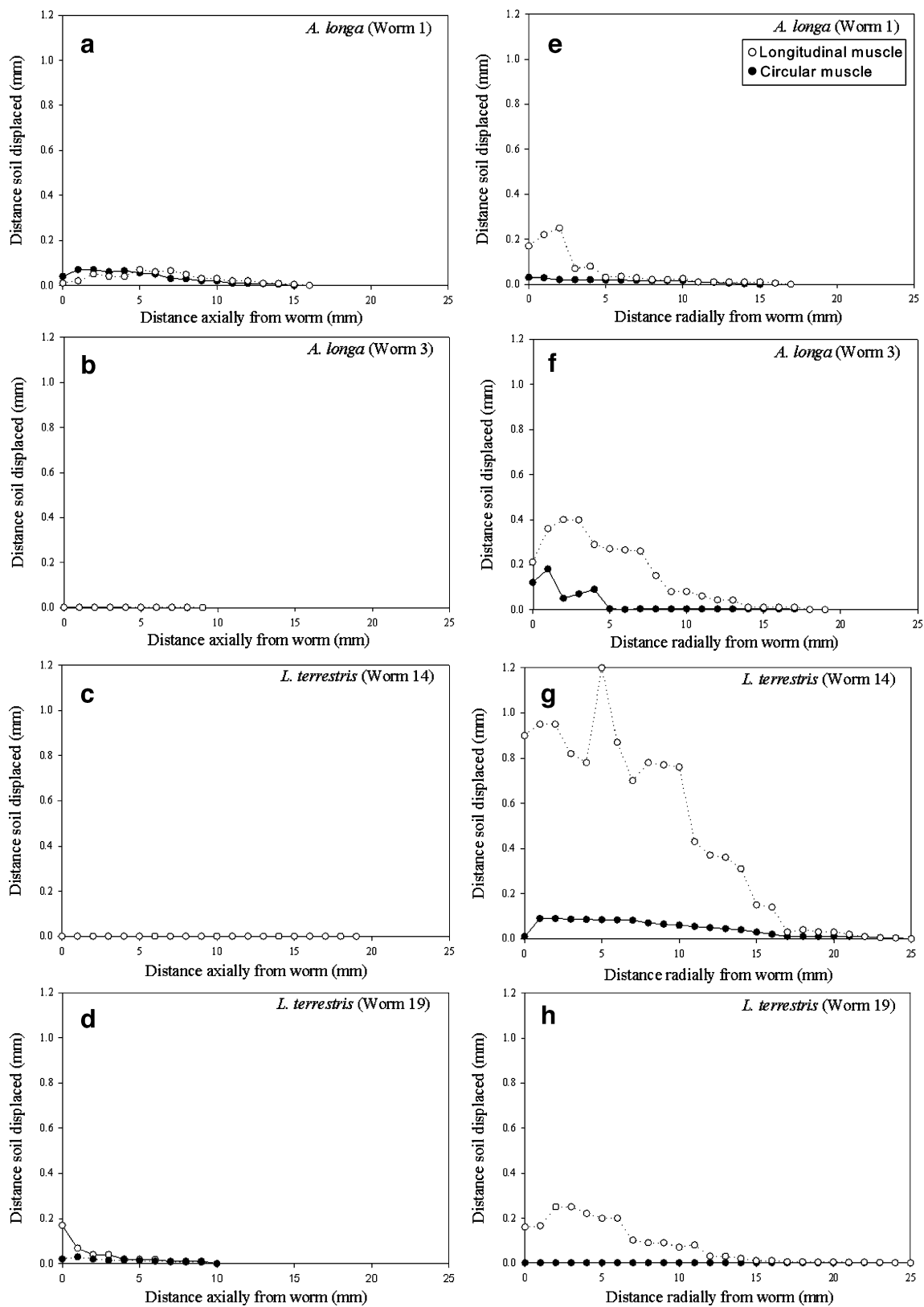
**Fig. 4** Vectors showing soil displacement adjacent to the earthworm's body. **a** *A. longa* and **b** *L. terrestris* image with its quiver plot (produced in Matlab) superimposed on top, showing patch (soil) displacement. Scale bar is 10 mm ( $n=6$  for *A. longa*;  $n=10$  for *L. terrestris*)



moving forward very slowly and often moved into voids, created by longitudinal contraction. Therefore, in some cases, very little soil displacement occurred during circular muscle contraction. Figure 5 gives an interesting insight into overall soil displacement, with cycling of large displacement of soil at the longitudinal contraction to small displacement at the circular muscle contraction.

The differences between species, the manner that burrows are formed (e.g. ingestion versus displacement), could lead to different conditions in their respective drilosphere. Movement of gasses over burrow walls result in differing aeration status, which will impact on the microbial interactions in the soil. In contrast to burrowing

in entirely natural conditions, these earthworms could only move in two dimensions. Whilst earthworms burrowing in similar systems have been shown to adopt normal burrowing behaviour (Schrader 1993) over periods of up to several weeks, whether short time scale behaviour is modified has yet to be determined. Particle image velocimetry quantifies the extent of soil displacement by burrowing and provides novel insights into the pattern of soil displacement caused by earthworm burrowing. To move from 2D to 3D would require rapid computer tomography scanning or similar that produced images suitable for displacement analysis in 3D. At the present time, scanning equipment is not sufficiently fast to take 3D images of earthworm movement.



**Fig. 5** Mean soil displacement at two different parts of the earthworm’s cycle of movement; the circular muscle contraction producing axial expansion (a–d) and the longitudinal muscle contraction producing radial expansion (e–h)

**Acknowledgements** We thank David White and Andy Take for access to the University of Cambridge PIV code and Stephen Hobson for help using the Matlab programme. We also thank Christine Hackett (Biomathematics and Statistics Scotland) for statistical advice. The Scottish Crop Research Institute receives grant-in-aid support from the Scottish Government Rural and Environment Research and Analysis Directorate.

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