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Microcosm investigations into the influence of sheep manure on the behaviour of the geophagous earthworms *Aporrectodea trapezoides* and *Microscolex dubius*

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Abstract A series of experiments was conducted over 96 h in 240-mm-deep soil microcosms, to assess the effect of the presence and distribution of sheep manure over the soil surface on the vertical and horizontal distribution of burrows and numbers of the earthworms *Aporrectodea trapezoides* and *Microscolex dubius*. Within some microcosms the dung was placed on half of the soil surface and this caused aggregation, with over two-thirds of the earthworms being found in the soil directly under the manure. The presence of surface-applied sheep manure caused both species to aggregate in the surface soil. In contrast, without manure, *A. trapezoides* was evenly distributed throughout the soil profile while *M. dubius* aggregated in the deeper soil. The pattern of burrow construction was also influenced by the presence of surface manure. In the absence of manure, burrows of both species were evenly distributed through the soil, but in the presence of surface manure *M. dubius* constructed proportionally more burrows close to the surface. Both species constructed approximately twice the burrow area in the absence than in the presence of surface manure. For both species the daily rate of burrow construction decreased over the experimental period. From these data we inferred that there was more widespread and active foraging behaviour in both species when organic food material was scarce. *M. dubius* differed from *A. trapezoides* in that it more strongly concentrated foraging activity in the vicinity of the manure food source.

Key words Earthworms · Burrowing behaviour · Food location · *Aporrectodea Microscolex*

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Introduction

Earthworms, through their burrowing, feeding and excretion, have the potential to promote soil fertility and plant productivity by improving water relations in soil, and by redistributing organic matter and speeding its mineralisation (Lee 1985). It is known that the addition of organic matter to the soil increases the number of earthworms and their activity, but little is known about how the location of organic matter influences the distribution and behaviour of earthworms.

In field studies, Hendriksen (1991) showed that in a Danish pasture *Lumbricus rubellus* aggregated under cattle dung, but *Aporrectodea caliginosa* did not, and Hughes et al. (1994a) showed four earthworm species had significantly higher densities in buried bags with sheep manure than in manure free bags, in a South Australian pasture. Evans (1947) used transparent microcosms to examine burrowing behaviour in more detail. He found that two British species of lumbricid earthworm constructed fewer burrows in the soil when there was manure on the soil surface than when there was no manure. With similar microcosms Martin (1982) showed three lumbricids introduced to New Zealand (including *A. trapezoides*) responded to decreased food concentrations in the soil by increasing soil consumption, and increasing the length of burrows constructed. In field plots in South Australia containing *A. trapezoides* and *Microscolex dubius*, addition of cereal straw decreased the number of surface-opening macropores per earthworm (Doube et al. 1994a). Thus there is evidence that organic matter affects both the distribution and the burrowing activity of earthworms, but there is little known about how the two are linked.

The geophagous earthworms *Aporrectodea trapezoides* and *Microscolex dubius* are common species in many temperate agricultural regions of the world, including southern Australia (Tisdall 1978; Lee 1985; McCredie and Parker 1991; McCredie et al. 1992; Baker et al. 1992; Buckerfield 1992; Lawson 1993; Doube et al. 1994a), where they occur primarily in the top 5–10 cm of the surface soil during moist seasons. They are both endogeic species which construct and backfill burrows in the surface soil during the moist season of the year. Both species grow rapidly if fed activated sewage sludge (McCredie and Parker 1991), but they can persist in field soils without added organic matter (Doube et al. 1994a), and can be relatively abundant in the presence of crop stubble or added cereal straw (Buckerfield 1992; Doube et al. 1994a, b). Their distributions within pastures can be very patchy, possibly in response to the patchy distribution of organic matter. In this paper we examine the relationships between the distribution of earthworms, their burrowing activity, and the presence of surface organic matter for the two species *A. trapezoides* and *M. dubius*.

Materials and methods

Experiments were conducted in microcosms within rectangular boxes, 400 mm long, 300 mm high and 25 mm wide, made from clear Perspex, with one side held in position with screws, and removeable. Each box was filled to 240 mm with 1200 g dried Urrbrae red-brown earth (1.2–1.3% carbon; Doube et al. 1994a), sieved through a 3-mm grid, and moistened with 160 ml distilled water. Dry soil was added to the boxes, 300 g at a time, and moistened and compacted with each addition to achieve an average bulk density of 0.5 g/ml. In the "with manure" treatment, 200 g moistened sheep manure (approx. 50% water by weight) was added to one-half of the soil surface to a depth of 10 mm, with bare soil left in the other half. In the "without manure" treatment, no manure was added. Each microcosm was sealed with plastic wrapping, and left for 1 week before adding earthworms. The microcosms were held in a 15°C room and were maintained in complete darkness under black plastic. Experiments were conducted in May 1991. There were six replicate trials with manure and two without manure for *A. trapezoides*, and four with manure and two without manure for *M. dubius*.

Earthworms were collected from field populations near Adelaide, South Australia. Ten adult earthworms, *A. trapezoides* weighing between 750 and 1150 mg, or *M. dubius* weighing between 550 and 750 mg, were added to each microcosm in a shallow pit in the centre of the soil surface. After 24 h the area of burrows visible through the sides of each box was traced onto transparent sheets. After 48 h and 96 h, new burrow area was also traced, using different colour pens. At 96 h, the side of each box was removed, and the location of each earthworm was recorded. We chose to terminate the experiment at 96 h because Doube et al. (1994c) had previously shown that *A. trapezoides* established a substantial burrow system in soft soil within that time.

For analysis, the microcosms were divided, either horizontally or vertically, and the mean number of earthworms or the mean burrow area per trial were compared between sections and treatments, using two-way analysis of variance.

Results

Vertical distribution of earthworms at 96 h

The locations of earthworms in the soil were allocated to three depth classes (0–8 cm, 8–16 cm, 16–24 cm) (Fig. 1). In the presence of surface-applied manure (the "with manure" treatment) both species of earthworm were significantly aggregated in the surface soil (*A. trapezoides* $F_{2,17}$=42.3, P<0.001; *M. dubius* $F_{2,11}$=37.8, P<0.001) with a high proportion (67% and 75% of *A. trapezoides* and *M. dubius*, respectively) occurring in the top 8 cm and a low proportion (10% and 3%, respectively, of *A. trapezoides* and *M. dubius*) at 16–24 cm soil depth (Fig. 1).

In the absence of manure ("without manure" treatment), no significant aggregation with soil depth was detected for *A. trapezoides* ($F_{2,5}$=1.2, P=0.42), whereas *M. dubius* was significantly aggregated ($F_{2,5}$=9.5, P=0.05) with 60% at 16–24 cm soil depth (Fig. 1). For both species, two-way analysis of variance showed significant treatment by depth interaction (*A. trapezoides* $F_{2,18}$=17.3, P<0.001; *M. dubius* $F_{2,12}$=32.8, P<0.001), indicating that the presence of surface manure radically altered their pattern of distribution with soil depth. With no manure most earthworms of both species were in the deepest soil, whereas the presence of manure on the surface led to more earthworms occurring close to the surface.

Vertical distribution of earthworm burrows at 96 h

In the presence of surface-applied sheep manure, there were significant differences between soil depths in the area of visible earthworm burrows for both species (*A. trape-*

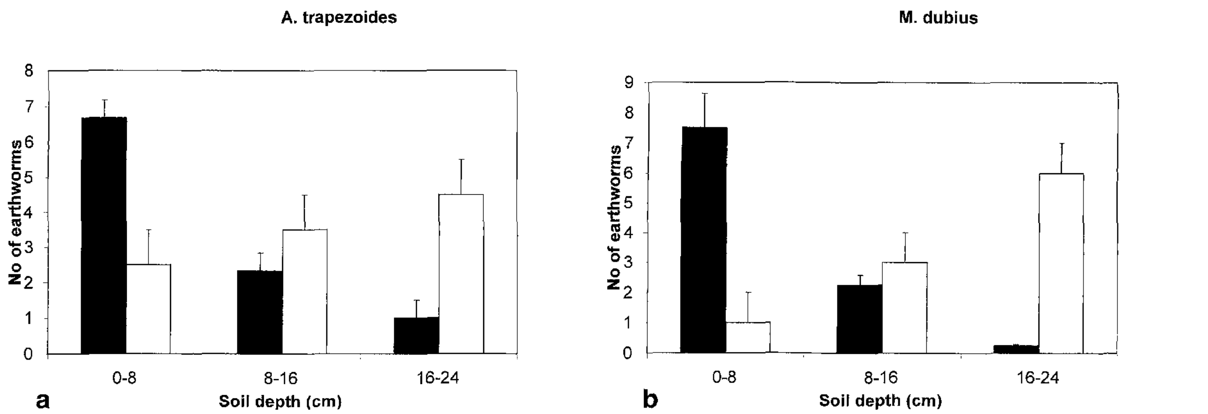

Fig. 1a, b Mean (+SE) number of earthworms in each depth class, after 96 h, in microcosms with (*filled bars*) and without (*empty bars*) manure applied to the surface

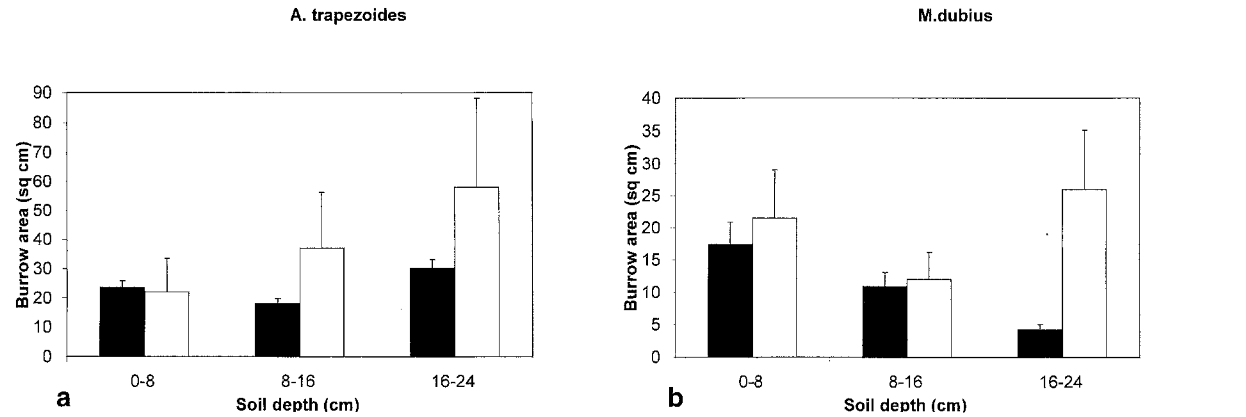

Fig. 2a, b Mean (+SE) total area (cm^2) of earthworm burrows visible through the sides of the experimental boxes in each depth class, after 96 h, in microcosms with (*filled bars*) and without (*empty bars*) manure applied to the surface

zoides $F_{2,17}$=4.9, P=0.02; *M. dubius* $F_{2,11}$=9.4, P=0.006) (Fig. 2). For *A. trapezoides* the greatest proportion (42%) was at 16–24 cm soil depth. Conversely, for *M. dubius* the greatest proportion of burrow area (54%) was in the surface layer and only 13% was in the deepest soil.

In the absence of manure, no significant effect of soil depth on the distribution of burrows was detected for either species (*A. trapezoides* $F_{2,5}$=3.1, P=0.19; *M. dubius* $F_{2,5}$=4.1, P=0.14) (Fig. 2). For *A. trapezoides* there was no significant treatment by depth interaction for the area of burrows ($F_{2,18}$=1.7, P=0.22), whereas the interaction was significant for *M. dubius* ($F_{2,12}$=22.3, P<0.001). The burrows for the latter species became more concentrated near the surface in the presence of surface manure.

The total area burrowed by *A. trapezoides* during the first 96 h was about double that of *M. dubius*, and within each species the area burrowed in the absence of manure was about double that in the presence of manure (Fig. 2).

Horizontal distribution of earthworms and burrows at 96 h

In the "with manure" treatment, the locations of earthworms in the soil were allocated to two classes based on the surface distribution of the manure (Fig. 3). The density of each species of earthworm was significantly greater (three-fold) in the soil under the manure than in the adjacent manure-free soil (Fig. 3a) (*A. trapezoides* $F_{1,11}$=32.6, P<0.001; *M. dubius* $F_{1,7}$=30.0, P=0.002). Similarly, for *M. dubius* there was twice the area of burrows under manure as under manure-free soil (Fig. 3b) ($F_{1,7}$=12.9, P=0.012). For *A. trapezoides* no significant difference in burrow area was detected between soil under manure and manure-free soil (Fig. 3b) ($F_{1,11}$=4.3, P=0.066).

Temporal sequence of burrow construction

The pattern of new burrow construction changed over the 96-h study period, varied between species and was affected by the presence of surface-applied manure (Fig. 4). Both *A. trapezoides* and *M. dubius* showed significantly less burrowing activity when manure was added ("with manure" treatment) than in the absence of manure ("without manure" treatment) (*A. trapezoides* $F_{1,137}$=28.5, P<0.001; *M. dubius* $F_{1,102}$=5.3, P=0.024). Both species also showed significantly less burrowing activity with increased time (*A. trapezoides* $F_{2,137}$=62.1, P<0.001; *M. dubius* $F_{2,102}$=20.8, P<0.001) (Fig. 4). For *A. trapezoides* there was a significant interaction between treatment and

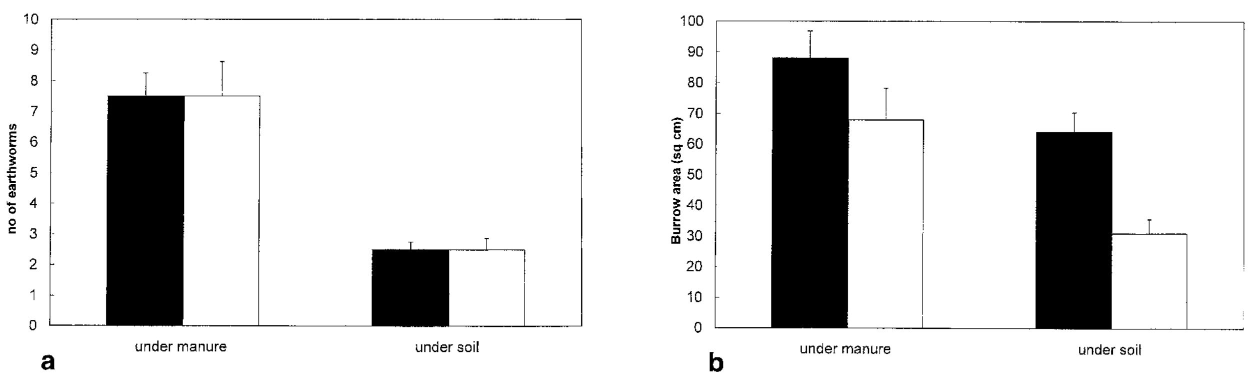

Fig. 3a, b For *A. trapezoides* (*filled bars*) and *M. dubius* (*empty bars*), the mean (+SE) number of earthworms and the mean (+SE) total area (cm^2) of earthworm burrows visible through the sides of the experimental boxes in soil under manure or under the adjacent bare surface. Data are all from the "with manure" treatment

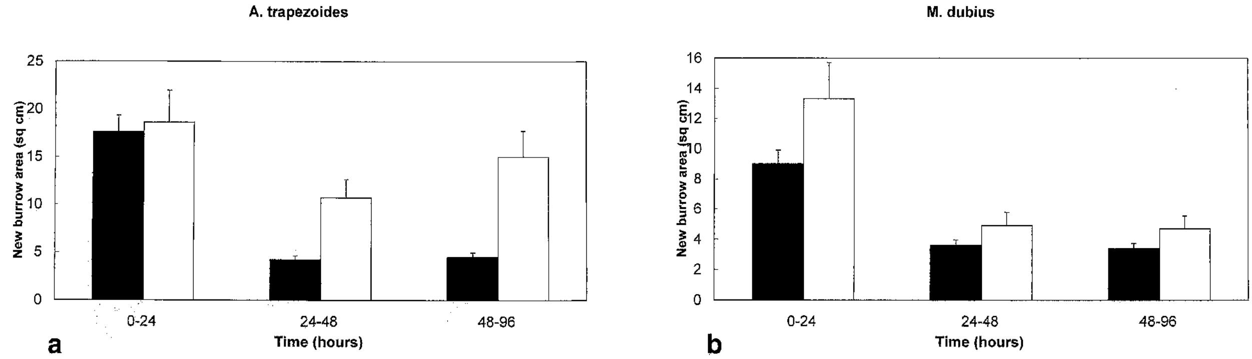

Fig. 4a, b Mean (+SE) new area (cm^2) of earthworm burrows visible through the sides of the experimental boxes after 24, 48 and 96 h with each earthworm species in microcosms with (*filled bars*) and without (*empty bars*) manure applied to the soil surface

time ($F_{2,137}$=5.9, P=0.003), resulting from earthworms reducing their burrowing activity with time less in the absence than in the presence of manure. There was no similar interaction effect for *M. dubius* ($F_{2,102}$=0.9, P=0.43).

Discussion

The two principal conclusions from these studies are, firstly, that the presence of sheep manure on the surface of the soil affected the behaviour of both species of earthworm, and secondly that the two earthworm species showed considerable differences in their behavioural responses to the presence of the surface organic matter.

Without manure the earthworms showed either a random distribution of locations in the soil profile (*A. trapezoides*), or a behavioural preference to be located in deep soil (*M. dubius*). This was reversed with manure applied to one-half of the soil surface, when both species were most commonly located in the surface soil. Earthworms of both species were located significantly more often in soil in the half under the manure. Sheep manure is a high-quality food for earthworms, and it promotes growth and development of both *M. dubius* (Hughes et al. 1994a, b) and *A. trapezoides* (Barley 1959; B.M. Doube, unpublished). Hendriksen (1991) showed that some (but not all) earthworm species aggregated under cattle dung in pastures. In field trials at Balhannah, South Australia, bags with sheep manure attracted 5 times as many *A. trapezoides*, and over 40 times as many *M. dubius*, as did control bags with no manure (Hughes et al. 1994a). The microcosm results reported in the present paper provide confirmation that both these earthworm species have a tendency to aggregate around a manure food source.

However, the two species showed substantial differences in their burrowing behaviour while responding to the added manure. Here we assume that the area of burrows visible through the side walls of each microcosm reflects the total amount of burrow constructed within the microcosm. *A. trapezoides*, the larger of the two species, produced more burrows than *M. dubius*. There was a non-significant trend for *A. trapezoides* to produce most burrows at the greatest soil depth when no manure was present. That trend was not significantly altered by the addition of manure to half of the soil surface. Furthermore, although manure covered only half of the soil surface, *A. trapezoides* burrowed as much under the bare soil as under the manure.

A. trapezoides appears to have a foraging strategy incorporating an extensive burrow system. It has previously been reported to carry surface organic matter such as canola petals (Doube et al. 1994b) and straw (Barley 1959) down into a burrow, rather than remaining at the food source. The burrowing may also be a response to natural conditions where ephemeral food sources become depleted and exploration for new sources is essential. When introduced to one end of horizontal tubes of soil, 21 cm long, *A. trapezoides* produced an even distribution of burrows within the tube after 8 days (Doube et al. 1994c).

M. dubius showed a different strategy. It significantly altered its burrowing behaviour in the presence of manure, concentrating its burrow construction close to the manure. This suggests that once it has located a food source, this species tends to remain close to that source, and concentrate its activities around it.

Both species burrowed more when there was no manure, in accord with the results of Evans (1947), Martin (1982) and Doube et al. (1994a). This may reflect a more active and widespread search for food by earthworms when food was scarce. In addition, where earthworms have a concentrated supply of organic matter (manure) they may reuse the burrow systems already established to exploit the food supply. Reuse of established burrows may also explain the decrease in new burrow construction with increased time. It is difficult to differentiate this explanation from a real decrease in earthworm activity over time. Of more interest is the temporal difference between treatments for *A. trapezoides*, which suggests that without manure, that species continues to construct new burrows and to forage actively for food. Martin (1982) also found that *A. trapezoides* constructed longer burrows when there was less organic food in the soil. *M. dubius* constructed smaller burrow area and fewer new burrows whether manure was or was not provided.

There is an anomaly in our results if earthworm distribution and burrow distribution are compared. The locations of earthworms imply that both species concentrate activity close to the food source. The locations of burrows suggest that *M. dubius* is active close to the food source, while *A. trapezoides* spreads its activity more broadly. We know little about diurnal activity patterns of earthworms in general (Lavelle 1988), or of these species in particular. However, a possible explanation of the anomaly is that our sampling of earthworm location was made during an active feeding phase. At other times, when they become inactive, *A. trapezoides* may retreat down its burrows away from the food source, while *M. dubius* may remain quiescent close to where it feeds.

These speculations suggest that further investigations using the microcosm may be rewarding. Although the microcosm contained soil packed more loosely than an earthworm may normally encounter, it provided detailed behavioural information, with non-destructive sampling, which would not normally be available from field surveys. The interspecific differences in response to the manure food source suggest the potential for subtle interactions between member species of the earthworm community.

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