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# Automated observation and analysis of earthworm surface behaviour under experimental habitat quality and availability conditions

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ABSTRACT

Currently, the driving factors of active earthworm dispersal across the soil surface are not sufficiently understood, and distances traversed by individual earthworms have rarely been quantified. Research progress has been hampered by the lack of adequate observation methods as well as fast, objective and quantitative measurements of nocturnal earthworm behaviour.

In this work, we report on the potential of a new, automated method using infrared-sensitive webcams and computer image analysis. Nightly surface activities of *Lumbricus terrestris* L. were monitored quantitatively while manipulating levels of disturbance, burrow availability and congener presence in standard observation units.

The automated observation system proved to be simple and inexpensive to build, provided reliable quantitative measures of locomotive behaviour without animal disturbance, and considerably reduced human workload and bias.

Waterlogging of the burrow zone stimulated surface activities around and away from the home burrow as compared to habitat disturbance by pesticide application and vibration. However, dispersing earthworms never settled in prefabricated burrows. Surface activity of individuals subjected to waterlogged conditions was influenced by the availability of alternative habitat. Minimal habitat disturbance (vibration) and the presence of conspecific individuals resulted in an increased amount of burrow-anchored, possibly sexually oriented, behaviour, but reciprocal burrow visits and mating were not observed. Pesticide application did not result in dispersal, increased foraging activities or sexual attraction attempts during this short-term study.

The presence, extent and mechanisms of the remote assessment of important cues in the surroundings of the home burrow by *L. terrestris* need further research. Infrared monitoring opens new research avenues of earthworm surface behaviour studies (e.g. density-dependent dispersal, habitat selection, (re)colonization, invasion).



## Introduction

Active dispersal of earthworms across the soil surface has long intrigued scientists but was seldom studied in detail after Darwin's (1881) early observations of mass emergence from burrows onto the soil surface during and following heavy rain. Dispersal may significantly influence temporal and spatial population dynamics (Valckx et al., 2009), thereby potentially altering the diversity, community structure, and functions of these ecosystem engineers in time and space. Dispersal is also a basic requirement of a successful (re)colonization of newly available habitats (Marinissen and Van den Bosch, 1992) and exotic species invasions (Bohlen et al., 2004).

Exogenous habitat quality deterioration (e.g. pesticide-induced migration, Christensen and Mather, 2004) and density-dependent regulation, often driven by endogenous habitat quality deterioration (Grigoropoulou and Butt, 2010), have been suggested as potential dispersal drivers. However, insights into the factors governing active horizontal surface dispersal are lacking, and distances traversed by individual earthworms have rarely been measured (but see Mather and Christensen, 1988).

The knowledge gap described above is at least partially related to the difficulties in devising appropriate observation methods that provide reliable quantitative data on dispersal behaviour and other nightly activities of these animals. Pioneering work

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by Nuutinen and Butt (1997) based on human and time-lapse video-assisted observation was recently modernized by Field and Michiels (2006) showing the potential of webcam monitoring in combination with infrared light. The latter researchers used manual post-processing on 30-s lapse images to quantify earthworm travel distance, residence time at the surface and number of surface visits. As both studies relied on manual image analysis, data collection was potentially subject to observer-induced bias and was very time consuming, thereby limiting the number of experiments that could be carried out.

Here we investigate the potential of a new, automated method using standard observation units, infrared-sensitive webcams and computer image analysis to objectively quantify the surface activity and dispersal behaviour of surface-active earthworm species under controlled experimental conditions. We report on the effects of exogenous habitat alteration, interaction with conspecific individuals and availability of stress-free habitat on the surface behaviour of the anecic earthworm *Lumbricus terrestris* L.

## Materials and methods

Earthworm behaviour was studied in a dark room where ambient air temperature fluctuated between 10.7 and 13.9 °C and relative air humidity varied between 52% and 87%. Fluorescent lamps (two above each observation unit, see below) provided a daily light period between 08.00 and 20.00 h. Observation units consisted of three parallel 0.1 m wide 'tracks', 1.2 m in length, and separated by 0.1 m high walls to prevent earthworm interaction and escape (Fig. 1). The tracks were filled to a height of 0.02–0.03 m with a loosely packed substrate of sandy soil (73% sand, 21% silt, 6% clay) with low organic matter content that had been sieved (4 mm) and was earthworm-free. Openings through the unit's base at 0.05 m from track ends allowed for connection to PVC tubes housing the focal earthworms or providing an alternative habitat (Fig. 1). Unused openings could be shut.

PVC tubes (0.5 m long, 0.05 m diameter) were prepared 2 days before experimental use. Tubes were filled with soil from the same stock as described above. The soil was tamped, and a vertical burrow was prefabricated in the centre of each tube by introducing a metal rod (diameter=0.005 m) through the entire soil column. Commercially obtained adult *L. terrestris* individuals (Eco-Cult, Roeselare, Belgium) were then weighed and introduced into as many tubes as required by the experimental setup. Tube bases were sealed with a fine mesh to prevent escape but to allow capillary water rising from the water reservoir in which the tubes were stored (0.05 m water level). Tubes were stored under the

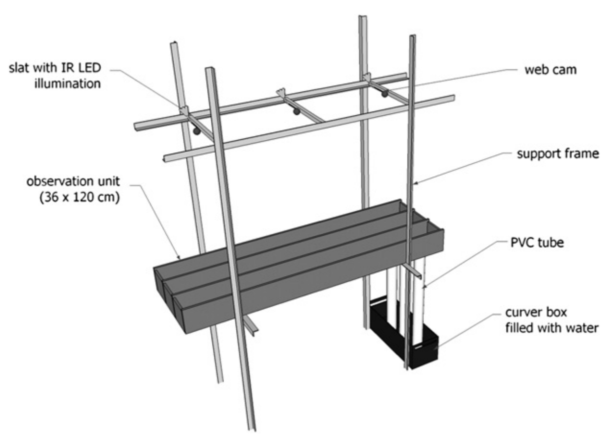


**Fig. 1**

same climatic conditions and light regime as described above. Earthworms were fed one piece of defrosted lettuce (1 $cm^2$) on the first day and each day thereafter if food from the previous day had been removed.

Three MSI Starcam 370i™ USB webcams equipped with a 640 × 480 pixel CMOS sensor with night vision capability were mounted at ca. 1 m height above each observation unit and connected to a PC. At this height, three cameras adequately captured the whole unit with sufficient detail and to allow some overlap between the fields of view of neighbouring cameras. Calibration marks were placed on the unit walls. Observation units were provided with customized uniform infrared LED illumination as it had already been demonstrated that red light and longer wavelength radiation have no effect on earthworm activity (Walton, 1927). The commercial JaxCam software (Jaxstream Inc., 2006) was programmed to save pictures in uncompressed jpeg-format every 30 s between 18.30 and 9.30 h. Exploratory observations showed that *L. terrestris* preferentially emerged during darkness, but some individuals deviated from this standard behaviour.

In a first series of experiments, the combined effects of local habitat quality alteration and remote habitat availability on the behaviour of *L. terrestris* were studied. Local habitat quality was manipulated by either (i) waterlogging the burrow of the focal earthworm, (ii) applying the herbicide Atlantis™, or (iii) applying mechanical vibrations to the PVC tube containing the focal earthworm. Waterlogging consisted of slowly pouring 0.2 l of tap water into the burrow inhabited by the focal earthworm, resulting in short-term surface ponding and a wetted soil surface after natural drainage that extended up to ca. 0.5 m away from the burrow entrance. Solutions of the herbicide Atlantis were prepared at the current farming practices concentration, diluted in 0.05 l of tap water (simulating water infiltration after rainfall; dilution factor 1:1275), and then slowly poured into the burrow thereby avoiding any surface water ponding. Control earthworms received 0.05 l of tap water in a similar way. In the vibration treatment Velleman™ solid-state buzzers (300–500 Hz) were fixed to the focal tubes and activated throughout the dark period. Remote habitat availability was varied (i) by providing no alternative habitat (burrow openings at the non-experimental manipulation end of each track were sealed), (ii) by providing a PVC tube with a non-inhabited prefabricated burrow at the other end of the track, or (iii) by connecting a PVC tube inhabited by an adult conspecific individual. In one control track per observation unit, habitat quality of the focal earthworm/tube was not manipulated while the remote habitat availability was modified as in the treated tracks. Treatments and controls were assigned randomly. All selected habitat quality × habitat availability treatments were tested during three consecutive nights. After each observation night, earthworms were recovered and weighed, and the observation units were cleaned with tap water and reassembled using fresh soil and earthworms.

As waterlogging proved to be a dispersal stimulus, an additional water gradient experiment was carried out in which 0.25 l of tap water was either applied to the focal or to the alternative habitat. Water in the focal burrow was either applied so that only the surrounding soil was wetted (as in the previous experiment), or so that the entire soil surface of the observation unit was wetted. Remote habitat availability was modified as in the previous experiment. The second experiment lasted for two nights before earthworms and soil substrates were replaced.

Automated image analysis, the details of which are reported elsewhere (Leroy T., unpublished data) allowed the calculation of a number of earthworm excursion and foraging-related variables. Briefly, individual earthworm movements were tracked based on the absolute differences between pairs of subsequent webcam