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SHORT COMMUNICATION

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Quantifying surface and subsurface cast production by earthworms under controlled laboratory conditions

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Abstract Earthworm casts, formed when organic substrates and soil minerals pass through the digestive tract, may protect soil organic matter from biological degradation if they persist in the soil. Yet, the stability of casts is affected by their location in the soil profile because surface casts are exposed to more disruptive forces (wetting-drying, freezing-thawing) than subsurface casts. It is not known whether environmental conditions affect the proportions of surface and subsurface casts produced by earthworms. This study investigated how surface and subsurface cast production by juveniles of *Aporrectodea* spp. and *Lumbricus* spp. was affected by temperature. Two juveniles of *Aporrectodea* spp. or *Lumbricus* spp. were added to plexiglass chambers filled with soil, and five replicate chambers were incubated in the dark at 5°C, 10°C, 15°C or 20°C for 1 week. Most of the casts produced by *Aporrectodea* spp. and *Lumbricus* spp. were surface casts, with <10% of casts deposited below the soil surface. The earthworms studied produced more casts, and a greater proportion of surface casts, as the soil temperature increased. These results can be used to estimate the quantity of surface and subsurface casts produced by earthworm populations under field conditions and determine the susceptibility of cast-associated organic matter to decomposition in the medium- to long-term.

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Introduction

Stable soil microaggregates are known to protect soil organic matter (SOM) from biological degradation and are important in regulating the size of SOM pools and global CO_2 flux. Earthworm casts, formed when organic substrates and soil minerals pass through the digestive tract, possess characteristics similar to microaggregates formed by other biological processes. Casts tend to be more stable to dispersive forces than microaggregates from the bulk soil, particularly in soils sensitive to physical disturbances, and contain more organic matter and higher levels of plant-available nutrients than bulk soil (Marinissen 1994; Schrader and Zhang 1997; Tomlin et al. 1995). Between 1 and 25.8 kg surface casts m^{-2} $year^{-1}$ are produced by earthworm populations in temperate regions (Binet and LeBayon 1999; Lee 1985), indicating that earthworms could influence the size of the microaggregate-protected SOM pool and alter SOM dynamics significantly.

The rate of SOM turnover from casts is affected by their location in the soil profile since surface casts are exposed to more disruptive forces (wetting-drying, freezing-thawing) than subsurface casts. Ge et al. (2001) recovered between 5% and 7% of casts placed on the soil surface and 74–81% of casts buried to a depth of 10 cm after 16 weeks, indicating that cast stability was preserved better in subsurface than surface casts. It is not known what proportion of the casts produced by earthworm populations are deposited on the soil surface or within the soil profile under field conditions, partly because no satisfactory method of measuring in situ cast production exists. Only a few earthworm species, namely *Lumbricus terrestris* L., *Aporrectodea caliginosa* (Sav.), *A. longa* (Ude) and *A. nocturna* (Sav.), produce surface casts (Edwards and Bohlen 1996; Scullion and Ramshaw 1988). In a tallgrass prairie, surface casts accounted for

<5% of the total casts produced by *Diplocardia* spp. and <2% of the total casts produced by Lumbricidae (James 1991). Therefore, methods that assess surface cast production only will probably underestimate the contribution of earthworm casts to SOM dynamics.

Cast production increases as temperature rises to an optimal level for earthworm activity (Bolton and Phillipson 1976; Daniel et al. 1996; Scheu 1987), but it is not know whether the proportion of casts deposited on the soil surface or within the soil profile changes at different temperatures. Knowledge of surface and subsurface cast production for different earthworm species under laboratory conditions could be used to estimate the quantity and stability of cast-associated organic matter generated by earthworm populations under field conditions. Such work would expand on the model of James (1991) that estimated total cast production for earthworms in a tallgrass prairie based on cast production rates at different temperatures in the laboratory and field measurements of temperature, soil moisture and earthworm populations.

The purpose of this study was to quantify the proportion and rate of surface and subsurface cast production by juveniles of *Aporrectodea* spp. and *Lumbricus* spp. as affected by temperature.

Materials and methods

Earthworm, soil and litter collection

Earthworms were collected by handsorting and formalin extraction in October 2002 from fields under alfalfa (*Medicago sativa* L.) production at the Macdonald Campus Farm, Ste-Anne-de-Bellevue, Québec, Canada. Earthworms of *Aporrectodea caliginosa*, *A. rosea* (Sav.), *A. tuberculata* (Eisen) and *L. terrestris* species were then reared (4 months) at 5°C in the original soil moistened to near field capacity. We also collected soil (fine-silty, mixed, frigid Typic Endoaquent), corn residues (leaves, stems and roots) and composted cattle manure for the study. Additional information on the soil and compost is reported in Carefoot and Whalen (2003).

Chamber design

Chambers were constructed of clear plexiglass sheets (45 cm high×45 cm wide). The distance between plexiglass sheets was 0.3 cm in chambers housing *Aporrectodea* spp. and 0.45 cm in chambers housing *Lumbricus* spp. This was achieved by placing a piece of plexiglass (5 cm wide by 0.3 or 0.45 cm thick) between the larger plexiglass sheets and inserting bolts (0.2 cm diameter) and nuts through holes drilled along the sides of the chamber. These dimensions permitted the earthworms to move freely and made it easy to visually observe and count subsurface casts. A sponge was inserted into the bottom 3 cm of the chamber and air-dried, sieved soil (<2 mm mesh) packed to a bulk density of about 1 g cm^{-3} was added to a height of 19 cm from the top of the chamber. Air-dried, sieved soil (<2 mm mesh) mixed thoroughly with 3 g corn shoots kg^{-1} soil, 1.5 g corn roots kg^{-1} and 6.5 g compost kg^{-1} was added between 4 and 19 cm from the top of the chamber (bulk density of about 1 g cm^{-3}), leaving the top 4 cm soil-free to monitor surface cast production. All litter was oven-dried (60°C, 48 h) and ground (<1 mm mesh), and the rates chosen approximate litter inputs in a conventionally tilled corn silage agroecosystem amended with 15 Mg compost (dry weight basis) ha^{-1} in this region (Carefoot and Whalen 2003). Soil was moistened to between 75% and 80% of field capacity and the chambers were incubated in the laboratory at 20°C in the dark for 1 week before adding the earthworms.

Surface and subsurface cast production

Juveniles of *Aporrectodea* spp. and *Lumbricus* spp. were placed on moistened tissue paper to void their gut for 24 h, weighed (fresh weight) and two individuals were added to each chamber. Individuals of *Aporrectodea* spp. weighed approximately 0.45 g and individuals of *Lumbricus* spp. weighed between 0.7 and 1.0 g. The top of the chamber was covered with duct tape containing small holes to allow air circulation but prevent the earthworms from leaving. Five replicate chambers for each species were placed in controlled climate incubators set at 5°C, 10°C, 15°C and 20°C (40 chambers in total). Surface and subsurface cast production in each chamber was assessed visually every 24 h and newly produced casts were marked with different coloured markers. After 1 week, the chambers were opened, the earthworms were removed and their fresh weights determined as above, and soil and surface cast samples taken for moisture content determination. Earthworm mass is expressed on a gram fresh weight (g f.w.) basis, while soil and cast weights are given on a gram dry weight (g d.w.) basis.

Statistical analysis

The effect of species (two levels), temperature (four levels), location of casts (two levels) and incubation time (repeated measures, seven levels) and the temperature×time interaction on cast production (no. casts g^{-1} earthworm f.w.) were evaluated by ANOVA in a general linear model (SAS for Windows, version 8.02). Data for repeated measures ANOVA did not show circularity. The main effects of species and cast location were significant ($P<0.001$) and data on subsurface and surface casting for *Aporrectodea* spp. and *Lumbricus* spp. are presented separately. The effect of temperature on the casting rate of each species was analysed by one-way ANOVA using data from day 4 to day 7 of the incubation to avoid laboratory artifice reported by Bolton and Phillipson (1976) and Scheu (1987). Mean comparisons of significant treatment effects were done with a Tukey-Kramer test at the 95% confidence level.

Results and discussion

Earthworm biomass declined by 5.3–7.0% for *Aporrectodea* spp. and by 0.6–3.8% for *Lumbricus* spp. (Table 1). This is a common occurrence in microcosm studies where earthworms must expend energy to construct burrows in unstructured soil and may be under stress due to handling and the experimental conditions (e.g., Bolton and Phillipson 1976).

Cast production varied significantly ($P<0.01$) with temperature and the observation time, with higher cast production at 20°C than other temperatures and more casts production on the first day of the incubation than subsequent days. Significant temperature×time interactions ($P<0.05$) are discussed below. The number of surface casts produced by *Aporrectodea* spp. was significantly greater on the first day than most other days, and did not differ between day 3 and day 7 of the incubation (Fig. 1). Subsurface cast production by *Aporrectodea* spp. was significantly greater in the first 2 days of incubation at 5°C, 10°C and 15°C than after day 3, but more subsurface casts were produced on day 3 of the incubation

Table 1 Effect of temperature on surface and subsurface cast production by *Aporrectodea* spp. and *Lumbricus* spp. under laboratory conditions. Values are means±SEM from day 4 to day 7 of the incubation (n=5). Values followed by the *same letter* within a column for each species are not significantly different at P<0.05 (Tukey-Kramer test). *f.w.* Fresh weight

Temperature (°C)	Initial earthworm mass[a] (g f.w. chamber^{-1})	Surface casts produced (no. chamber^{-1})		Subsurface casts produced (no. chamber^{-1})		Surface cast production (mg g^{-1} day^{-1})	
Aporrectodea spp.							
5	0.86±0.02	6±2	d	0	a	9.6±3.4	d
10	0.90±0.02	18±3	c	0	a	31.0±4.8	c
15	0.90±0.01	36±3	b	0.4±0.4	a	61.5±4.8	b
20	0.89±0.01	82±4	a	0.4±0.4	a	142.2±5.5	a
Lumbricus spp.							
5	1.30±0.06	24±4	c	3±1	b	32.2±3.7	c
10	1.32±0.07	39±4	c	4±1	b	53.9±6.6	bc
15	1.81±0.24	65±4	b	3±1	b	68.3±7.7	b
20	1.48±0.11	121±11	a	8±1	a	158.3±11.4	a

[a] Two juveniles per chamber

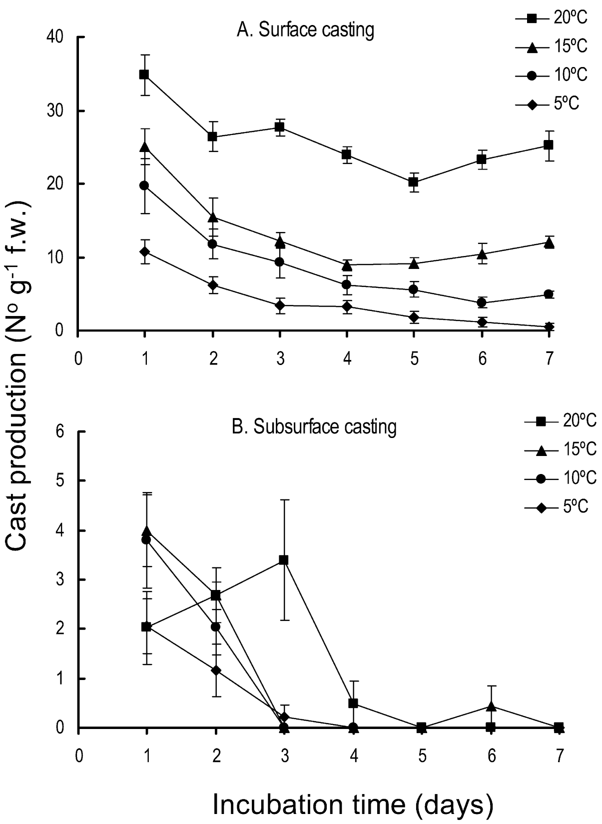

Fig. 1 Surface (**A**) and subsurface (**B**) cast production [no. casts g^{-1} earthworm fresh weight (*f.w.*)] by immature *Aporrectodea* spp. during a 7-day incubation at 5, 10, 15 and 20°C (means±SEM; n=5)

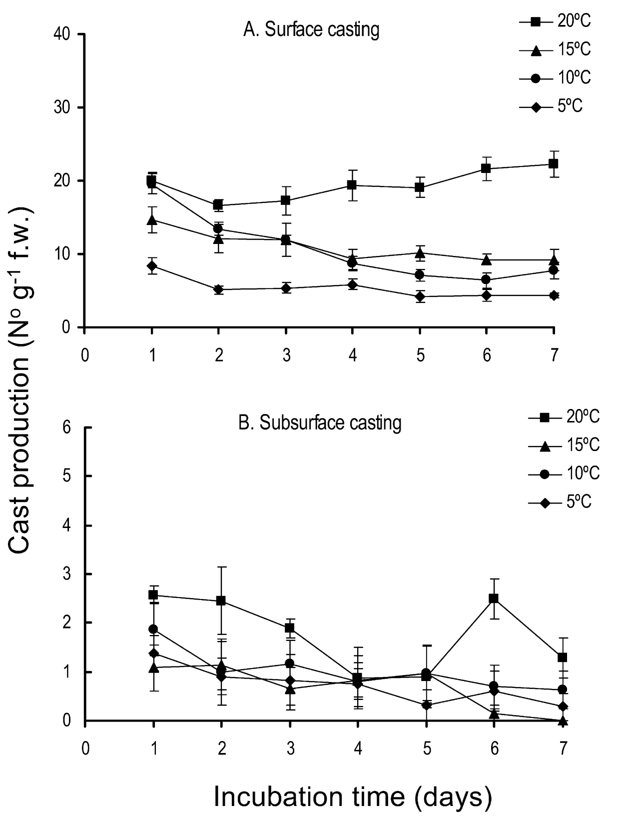

Fig. 2 Surface (**A**) and subsurface (**B**) cast production (no. casts g^{-1} earthworm f.w.) by immature *Lumbricus* spp. during a 7-day incubation at 5°C, 10°C, 15°C and 20°C (means±SEM; n=5)

at 20°C than on other dates (Fig. 1). The number of surface casts produced by *Lumbricus* spp. declined significantly after the first day of incubation at 5°C and 10°C, and did not differ between day 4 and day 7 of the incubation (Fig. 2). There was no change in surface cast production by *Lumbricus* spp. incubated at 15°C and 20°C or subsurface cast production during the incubation (Fig. 2).

When *A. rosea*, *A. caliginosa* and other earthworm species are introduced into laboratory chambers containing sieved unstructured soil, they spend the first 2–3 days constructing burrows, leading to an elevated rate of cast production (Bolton and Phillipson 1976; Scheu 1987). We observed a similar increase in cast production by *Aporrectodea* spp. in the first 3 days of the incubation, but the experimental conditions did not consistently induce cast production by *Lumbricus* spp.

Most of the casts produced by *Aporrectodea* spp. and *Lumbricus* spp. were surface casts; only 10% or fewer of the total casts produced were deposited below the soil surface (Table 1). The weight of surface casts produced by *Aporrectodea* spp. (6.8±0.1 mg d.w. g^{-1} earthworm f.w.) and *Lumbricus* spp. (5.1±0.2 mg d.w. g^{-1} earthworm f.w.) was unaffected by incubation temperature. More surface casts were produced by *Aporrectodea* spp. and *Lumbricus* spp. at 20°C than at other temperatures, and more subsurface casts were produced by *L. terrestris* at 20°C than other temperatures (Table 1). Surface cast production (SCP, no. g^{-1} f.w.) increased with increasing soil temperature (T, °C) and was described by the following linear relationships:

$$\mathrm{SCP} = 3.97\ T - 7.4 \quad \textit{Aporrectodea}\ \text{spp.}, \quad R^2 = 0.750, \ \ n = 20 \tag{1}$$

$$\mathrm{SCP} = 5.55\ T - 29.4 \quad \textit{Lumbricus}\ \text{spp.}, \quad R^2 = 0.879, \ \ n = 20 \tag{2}$$

It is well established that cast production increases as temperature rises to an optimal level for earthworm activity. Bolton and Phillipson (1976) measured higher rates of casting by *A. rosea* at 14.8°C than 10°C or 4.4°C, while *A. caliginosa* produced 3–4 times more casts at 15°C than 5°C (Scheu 1987). A model of cast production developed by Daniel et al. (1996) indicated that the maximum rate of cast production by *A. nocturna* occurred at 16°C. Our results are consistent with published reports, but indicate that cast production increases beyond 15–16°C for juveniles of *Aporrectodea* spp. and *Lumbricus* spp. Our results also indicate that a greater proportion of total casts are deposited on the soil surface as the soil temperature increases. These relationships should be verified under field conditions before we speculate on how casting activity may influence SOM dynamics.

Surface cast production ranged from 9.6 to 142.2 mg cast d.w. g^{-1} earthworm f.w. day^{-1} for *Aporrectodea* spp. and from 32.2 to 158.3 mg g^{-1} day^{-1} for *Lumbricus* spp. (Table 1). Our values for *Aporrectodea* spp. tend to be lower than cast production rates of 200–4,400 mg g^{-1} day^{-1} reported for *A. caliginosa*, *A. longa*, *A. rosea*, *A. trapezoides* (Eisen) and *A. longa* (Barley 1959; Bolton and Phillipson 1976; Böström 1988; Curry and Baker 1998; Scheu 1987). Cast production rates for *Lumbricus* spp. were similar to the 70–500 mg g^{-1} day^{-1} reported for *L. terrestris* (Buck et al. 1999; Satchell 1967; Shipitalo et al. 1988). Under laboratory conditions, cast production is affected by factors such as the earthworm species, the stage of development, temperature, soil moisture, and the quantity and type of food provided (Bolton and Phillipson 1976; Buck et al. 1999; Shipitalo et al. 1988). In addition, the chambers that we used were larger than chambers based on the design of Evans (1947) that are commonly used to measure cast production rates. Further study is needed to determine whether rate and distribution of casts produced by *Aporrectodea* spp. and *Lumbricus* spp. under controlled conditions accurately represents their activity in the field.

In conclusion, we found that the chambers used in this study permitted visual assessment of surface and subsurface cast production without disturbing the earthworms, making the design practical and easy to use. Earthworms of *Aporrectodea* spp. and *Lumbricus* spp. produce more casts, and a greater proportion of surface casts, as the soil temperature increases. Most of the casts produced were deposited on the soil surface. These findings should be verified under field conditions, allowing us to estimate the quantity of casts produced by earthworm populations and determine how earthworm casts may contribute to the microaggregate-protected SOM pool in the medium- to long-term.

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