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Original article

The "*deduction*" approach: A non-invasive method for estimating secondary production of earthworm communities

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ABSTRACT

Secondary production is an important parameter for the study of population dynamics and energy flow through animal communities. Secondary production of earthworm communities has been determined with the size-frequency and instantaneous growth rate methods, whereby earthworm populations are repeatedly sampled at regular intervals and the change in biomass of cohorts or individuals between sampling dates is determined. The major disadvantage of repeated sampling is that it disturbs the soil and permanently removes earthworms from the study area. The "*deduction*" approach is a theoretical model that partitions individuals into defined pools and makes assumptions about the growth, recruitment and mortality of each pool. In 2004 and 2005, earthworms were added to undisturbed field enclosures and the "*deduction*" approach was used to estimate secondary production of the indigenous and added earthworm populations during the crop growing period (17–18 weeks) in each year. Secondary production estimates made by the "*deduction*" approach is an indirect method that estimates population dynamics and secondary production, and is appropriate for manipulation experiments where removal of organisms and physical disturbance of the habitat by repeated sampling could bias results.

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1. Introduction

Secondary production describes the growth, mortality and recruitment of individuals in a population and is often used to estimate the energy flow in aquatic food webs (Benke, 1984). Both direct and indirect methods of estimating secondary production have been developed. Calculations of secondary production using direct methods such as the cohort method (Crisp, 1971), sizefrequency method (Hynes, 1961), and the instantaneous growth rate method (Romanovsky and Polishchuk, 1982) generally yield comparable results (Medernach and Gremare, 1999; Sardá et al., 2000). Indirect methods for estimating secondary production are based on empirical relationships between body size and production, but do not give similar estimates of secondary production unless a large population size and a broad range of environmental conditions are used to develop the relationships (Sardá et al., 2000).

Secondary production is also relevant to terrestrial organisms such as earthworms. Due to their key influence on soil organic matter decomposition, nutrient cycling and primary production (Fragoso et al., 1997; Lavelle et al., 1997), many researchers have attempted to quantify energy and nitrogen flux through earthworm communities from secondary production estimates (Parmelee and Crossley, 1988; Whalen and Parmelee, 2000). Secondary production represents about 8–19% of the N cycled by earthworm communities, which is estimated at between 7 and 363 kg N ha⁻¹ year⁻¹ (Parmelee and Crossley, 1988; Marinissen and de Ruiter, 1993; Curry et al., 1995; Whalen and Parmelee, 2000). This is an important contribution to N cycling, considering that the annual N demand of field crops (wheat, maize, etc.) often exceeds 100 kg N ha⁻¹.

The direct methods of calculating secondary production in earthworm communities involve sampling earthworm populations at regular intervals throughout the frost-free periods of the year and inferring the change in earthworm biomass between sampling dates (Satchell, 1963; Boström, 1988; Parmelee and Crossley, 1988). There is considerable variation in secondary production estimates, even at a single field site, due to difficulties in gauging the age of individuals, the lack of cohort-specific developmental phases and the heterogeneous distribution of naturally-occurring communities (Rossi et al., 1997; Whalen and Parmelee, 2000). Earthworm manipulation experiments permit researchers to add individuals of known age and biomass to a designated area (enclosure), and

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determine their impact on soil properties and plant growth during a period of time (Eriksen-Hamel and Whalen, 2007a). Intact laboratory mesocosms and undisturbed field enclosures favor the survival and growth of earthworms added in manipulation experiments, but the major drawback is that we cannot assess the earthworm population (cocoons, juveniles and adults) already inhabiting the experimental unit. Another difficulty is that regular sampling of earthworm populations in the mesocosm or enclosure is not possible because it would disrupt the soil habitat and remove earthworms from the experiment. Since direct methods of calculating secondary production cannot be used in earthworm manipulation experiments, we developed the "deduction" approach, which permits researchers to estimate the population dynamics of earthworms and other cryptic animals without repeated invasive sampling. The earthworm population (number of individuals and biomass) is measured only once, at the end of the experiment, and the "deduction" approach is used to infer the initial biomass. Then, secondary production is calculated as the difference between the initial and final biomass minus estimated mortality and recruitment. The "deduction" approach does not contradict direct methods of estimating secondary production that are based on stochastic, stage-based Leslie matrix population models (Tondoh, 1998; Svendsen et al., 2005; Pelosi et al., 2008), survivorship curves or growth rates (Lakhani and Satchell, 1970; Boström, 1988; Parmelee and Crossley, 1988). However, the "deduction" approach does not require information on the initial earthworm population (i.e., the number or biomass of cocoons, juveniles, sub-adults and adults at the beginning of the study) or the progression of individuals through age-classes, which are needed in population models. Therefore, the "deduction" approach is an indirect method that can deduce the change between initial and final age-class from life-history parameters, thus providing insight into population dynamics and secondary production of organisms.

The objectives of this paper were: (1) to use the "*deduction*" approach to estimate secondary production of earthworm communities in field enclosures; and (2) compare our estimates to earthworm secondary production values obtained in other published studies. This was based on assumptions about growth, recruitment and mortality of an introduced earthworm community during a 17 week period (June–October) in soybean and maize agroecosystems.

2. Materials and methods

2.1. Description of field site and experiment design

A field experiment was designed to evaluate the effects of controlled earthworm populations on soil properties and crop yield in field enclosures. It was conducted from May 2004 to September 2005 on the Macdonald Campus Research Farm, McGill University, Quebec, Canada (45°25′ N, 73°56′ W). In May 2004, rectangular sheet metal field enclosures, measuring $2.4 \text{ m} \times 1.2 \text{ m} (2.9 \text{ m}^2)$, were buried to a depth of 0.30-0.40 m leaving 0.1 m high enclosures above soil. The corners and top edges of the enclosures were bent at right angles to ensure a tight fit between pieces and minimize earthworms escaping from the enclosures (Eriksen-Hamel and Whalen, 2007a). Seven enclosures were replicated in four blocks, for a total of 28 experimental units. During the months of May 2004 and May 2005, carbaryl pesticide (Sevin[®]) was applied four to five times to each enclosure (total application of 220 kg a.i. $ha^{-1} year^{-1}$) to reduce earthworm populations. The pesticide was applied according to recommended applications rates made by Potter et al. (1990). Native earthworms collected from the surrounding field were added to the enclosures on June 1, 2004 and on June 6, 2005, and remained in the enclosures for about 17-18 weeks. The seven treatments included three earthworm populations as Aporrectodea caliginosa only (Ac), Lumbricus terrestris only (Lt), and a combined A. caliginosa and L. terrestris treatment (AcLt), at either the background population level $(1 \times)$ or double the background population level $(2\times)$, and a control treatment (Control) (Table 1). Earthworms were sampled from a soil pit $(50 \times 30 \text{ cm to a depth of } 20 \text{ cm})$ dug in the middle of each enclosure on September 30, 2004 and September 28, 2005. Formalin extraction (Raw, 1959) was used to collect earthworms from lower depths beneath the pit. Earthworm numbers, ageclasses, formalin-preserved biomass and ash-free dry weight (AFDW) of collected earthworms were later recorded in the lab. Ash-free dry weights were determined by placing dried (90 °C for 24 h) earthworms in a muffle furnace at 500 °C for 4 h. Sexually mature individuals were identified to the species level using the key provided by Reynolds (1977). The number and biomass of earthworms added in each treatment in the spring and collected in the autumn are provided in Table 1. Further details of the experimental design, description of soils, and methods of plant and soil sampling and analysis are described in Eriksen-Hamel and Whalen (2007a).

2.2. Assumptions about earthworm activity

The number of days that earthworms are active throughout the year significantly impacts the estimates of secondary production. Based on weekly soil temperature and moisture measurements from each enclosure (Fig. 1), the growth rates of *A. caliginosa* in soil from the site (Eriksen-Hamel and Whalen, 2006) and casting activity of earthworms in enclosures (Perreault et al., 2007), we determined that earthworm growth and activity ceased when soil temperatures exceeded 22 °C and soil moisture was below 20% WFPS. Thus, earthworm activity was positive (+) at soil temperature <22 °C and soil moisture >20% WFPS, while neutral (0) activity occurred at these values and negative (-) activity occurred

Table 1

Earthworm numbers (ind. m^{-2}) and fresh weight biomass (g fw m^{-2}) added in June (A_i), mean biomass collected in September (Treatment_{final}), and the mean active biomass (B_{mean}) in each treatment in 2004 and 2005. 1: Ac = A. caliginosa; Lt = L. terrestris; x1 = natural population level; x2 = double the natural population level.

| Earthworm Treatment ¹ | 2004 | | | | 2005 | | | |
|----------------------------------|--------------------------|---------------|----------------------------|-------------------|------------------------------------|----------------------|------------------------------|-------------------|
| | Earthworms added (A_i) | | Treatment _{final} | B _{mean} | Earthworms added (A _i) | | Treatment _{final} | B _{mean} |
| | Ind. m ⁻² | g fw m^{-2} | g fw m^{-2} (±S.E.) | | Ind. m^{-2} | g fw m ⁻² | g fw m ⁻² (±S.E.) | |
| 1: Control | 0 | 0 | 25 ± 7.5 | 20 | 0 | 0 | 23 ± 5.4 | 19 |
| 2: Acx1 | 50 | 21 | 56 ± 15 | 53 | 50 | 24 | 34 ± 7.1 | 42 |
| 3: Acx2 | 100 | 42 | 86 ± 22 | 75 | 100 | 47 | 25 ± 8.4 | 46 |
| 4: Ltx1 | 15 | 34 | 55 ± 9.2 | 56 | 15 | 37 | 44 ± 19 | 55 |
| 5: Ltx2 | 30 | 67 | 77 ± 12 | 85 | 30 | 75 | 33 ± 10 | 66 |
| 6: AcLtx1 | 65 | 55 | 86 ± 25 | 97 | 65 | 61 | 43 ± 11 | 65 |
| 7: AcLtx2 | 130 | 109 | 92 ± 8.7 | 127 | 130 | 123 | 44 ± 20 | 93 |



Fig. 1. Mean weekly soil temperature (°C) (gray and solid line) and water filled pore space (%) (black and dashed line) in enclosures in 2004 and 2005. The gray long dashed line at 22 °C and black dotted line at 20% indicate the limits of earthworm growth. Periods of positive and negative earthworm activity are shown for each week.

when the critical temperature and moisture levels were exceeded (Fig. 1). Although earthworms were expected to be inactive on days with negative activity, we assumed that earthworms could possibly be active on days with neutral activity. We estimated the number of days with positive earthworm activity was 77 (\pm 14) in 2004 and 49 (\pm 14) in 2005, where the uncertainty associated with these values is the number of days with neutral activity (14 d) in each year (Fig. 1, Table 2).

2.3. Assumptions about earthworm growth and mortality to estimate earthworm secondary production

Earthworm numbers and biomass in each enclosure changed between the date of earthworm addition (early June) and the final population assessment following crop harvest (Table 1). Most lateral movement by earthworms occurs in the 0–20 cm depth (Francis et al., 2001; Bastardie et al., 2003) and since the enclosures extended 30–40 cm deep, we assumed that there was no immigration or emigration from the enclosures. We also assumed that earthworm removal by predation (e.g. birds) was negligible in all enclosures. Bird flocks were observed often on neighboring alfalfa fields, which typically have large earthworm populations, and we presume that foraging success was greater elsewhere than at our field site. Removal of surface residues in the spring and regular weeding probably made field enclosures unattractive for other earthworm predators, as we saw no evidence of voles or snakes in the vicinity of our study site. Therefore, the fluctuations in earthworm populations and biomass were due to recruitment of

Table 2

Parameter values used in the "*deduction*" approach for estimating secondary production of earthworm communities in 2004 and 2005. 1: Ac = A. caliginosa; Lt & Al = L. terrestris and A. longa.

| Parameter | Estimate | | References |
|---|--------------------------------|-----------------|---|
| Active period (non-aestivation) | 2004: 77 days 2005: 49 days | | Fig. 1; Eriksen-Hamel and Whalen, 2007 |
| Mortality during season $(M_{\rm S} + M_{\rm C} + M_{\rm R1} + M_{\rm R2})$ | 35% | | Al-Yousef and Shoreit, 1992; Wever et al., 2001; Eriksen-Hamel and Whalen, 2007 |
| Mortality of added earthworms (M_A) | 50% | | Subler et al., 1997; Boyer et al., 1999; Eriksen-Hamel and Whalen 2006, 2007 |
| Instantaneous growth rate (d^{-1}) | Ac Lt & Al | 0.006 0.0008 | Whalen and Parmlee, 1999; Zwhalen et al., 2003; Eriksen-Hamel and Whalen, 2006 |
| g fw: g AFDW | 7.5 | | Experimental observations, Boström, 1988; Curry et al., 1995 |

hatchlings from cocoons, growth (secondary production) and biomass lost via mortality.

The "*deduction*" approach involves assigning earthworms to defined pools and deducing the fate of each pool with assumptions about growth, reproduction and mortality (Fig. 2). The earthworm biomass (g fw m⁻²) in the control treatment at the end of the season (Control_{final}) was from earthworms that survived pesticide application (S_f), earthworms that hatched from a cocoon deposited prior to pesticide application (C_f) and the offspring of these earthworms (R1).

$$Control_{final}(g \text{ fw } m^{-2}) = S_f + C_f + R1$$
(1)

To differentiate these pools ($S_{\rm f}$, $C_{\rm f}$ and R1), we determined the maximum biomass of an earthworm that hatched from a cocoon the day after the last pesticide application. This was based on the number of days with positive earthworm activity (77 d in 2004, 49 d in 2005) and growth curves for each earthworm species obtained from the literature (Table 3). We used growth curves to estimate the growth of newly emerged earthworms because instantaneous growth rates describe the logistic growth of juvenile or adult earthworms and would have underestimated the rapid linear growth of newly emerged earthworms. A newly emerged earthworm of any species could grow to 0.4 g fw during the study period in 2004 and to 0.3 g fw in 2005 (Table 3). Thus, the $S_{\rm f}$ pool was the sum of the biomass of all individuals weighing more than these values.

The biomass of earthworms in treatment enclosures at the end of the season (Treatment_{final}) was calculated from Eq. (2).

$$Treatment_{final}(g fw m^{-2}) = Control_{final} + Treatment_{added},$$
 (2)



Fig. 2. Flowchart of the earthworm population dynamics in control and treatment enclosures used for the calculation of secondary production using the "*deduction*" approach.

The assumption was that Control_{final} biomass was representative of earthworms surviving the pesticide application and their offspring in all enclosures; as well *A. longa* collected from treatment enclosures were allocated to the Control_{final} pool since this species was not added to enclosures. The Treatment_{added} pool was the biomass at the end of the season of earthworms added to enclosures (A_f) and their offspring (R2).

$$Treatment_{added} (g fw m^{-2}) = A_f + R2$$
(3)

Earthworm biomass was partitioned between the A_f and R2 pools based on the number of days with positive earthworm activity and instantaneous growth rate (IGR) values obtained from the literature (Table 2). The initial biomass (B_i , g fw) of *A. caliginosa* juveniles added to enclosures (A_i) were 0.31 g \pm 0.01 (S.E.) in 2004 and 0.23 g \pm 0.01 in 2005, while *L. terrestris* juveniles weighed 1.5 g \pm 0.10 in 2004 and 1.9 g \pm 0.11 in 2005. We calculated the final biomass (B_f) for each earthworm species using a logarithmic growth rate equation (Eriksen-Hamel and Whalen, 2006).

$$B_{\rm f}({
m g\,fw}) = B_i \times \exp\left[{
m active \ days \ (d)} \times {
m IGR}\left(d^{-1}\right)
ight]$$
 (4)

where IGR is the instantaneous growth rate and active days were the number of days with positive earthworm activity (Table 2). We calculated that *A. caliginosa* would grow to a minimum biomass of 0.45 g in 2004 and 0.35 g in 2005, while *L. terrestris* were expected to grow to 1.6 g in 2004 and 2.0 g in 2005. Earthworms weighing more than the minimum biomass were considered in the A_f pool, while earthworms weighing less than this critical level were considered as being recruited from A(R2) pool.

Earthworm secondary production (*P*) was biomass accumulated from all pools between earthworm addition and final biomass measurement.

$$P(g \text{ fw } m^{-2}) = \Delta S + \Delta A + C_{f} + R1 + R2 + M_{t}$$
(5)

$$\Delta S(gfwm^{-2}) = S_f - S_i = S_f \times [1 - 1/(exp(active days \times IGR))]$$
 (6)

$$\Delta A(gfwm^{-2}) = A_f - A_i = A_f \times [1 - 1/(exp(active days \times IGR))]$$
 (7)

where ΔS and ΔA is the biomass accumulated in the *S* and *A* pools. The difference between initial and final biomass in both the *S* and *A* pools was calculated from Eq. (4). Earthworm mortality (*M*_t) from each enclosure during the study period was the sum of mortality from all pools.

$$M_t(g \text{ fw } m^{-2}) = M_S + M_C + M_A + M_{R1} + M_{R2},$$
 (8)

Mortality of naturally-occurring (M_S, M_C) and recruited (M_{R1}, M_{R2}) earthworms was estimated to be 35% of the earthworm biomass in

Table 3

Maximum biomass (g fw) of an earthworm that hatched from a cocoon the day after the last pesticide application. Values for each species were obtained from growth curves and growth rates reported in the literature.

| Earthworm species | | 2004 (77 days) | 2005 (49 days) | References |
|-------------------|------------|----------------|----------------|--|
| A. caliginosa | Literature | 0.30-0.80 | 0.20-0.50 | Lofs-Holmin, 1982; Boström and Lofs-Holmin, 1986; |
| | This study | 0.4 | 0.3 | Whalen and Parmelee, 1999 |
| L. terrestris | Literature | 0.3-1.2 | 0.20-0.6 | Butt, 1991; Whalen and Parmelee, 1999; Lowe and Butt, 2003 |
| | This study | 0.4 | 0.3 | |
| A. longa | Literature | 0.4-1.3 | 0.30-0.8 | Butt, 1998; Lowe and Butt, 2002; Baker and Whitby, 2003 |
| | This study | 0.4 | 0.3 | |

each pool (*S*, *C*, *R*1, *R*2) (Table 2). Mortality of added earthworms (*M*_A) was estimated to be 50% of the *A*_f pool (Table 2). The mortality of added earthworms was assumed to be higher than the mortality of naturally-occurring earthworms (*M*_S, *M*_C) because of the stress involved in handling and adding earthworms to the enclosures. Mortality was based on mortality rates that occur under high temperatures (>20 °C) and low soil moisture conditions (<-30 kPa) in laboratory cultures and field experiments (Table 2).

2.4. Calculation of mean earthworm biomass during the season

The mean biomass (B_{mean}) of active earthworms in the enclosures during the growing season was calculated by Eqs. (9)–(11):

 B_{mean} in control enclosures (g AFDW m⁻²)

$$= \left(S_{i} + \text{Control}_{\text{final}}\right)/2, \tag{9}$$

 B_{mean} in treatment enclosures (g AFDW m⁻²)

$$= \left(S_{i} + A_{i} + \text{Treatment}_{\text{final}}\right)/2, \tag{10}$$

$$S_i \left(g \text{ AFDW } m^{-2} \right) = S_f / e^{[active \ days \times IGR]}$$
 (11)

where A_i is the initial biomass of earthworms added to each treatment at the beginning of the experiment (Table 1), S_i and S_f are the initial and final biomass of earthworms surviving pesticide application.

2.5. Comparison of measured vs estimated values of A_i

We evaluated the assumptions of growth, reproduction and mortality used in the "*deduction*" approach by comparing the measured biomass of earthworms added to enclosures (A_i) with the biomass estimated by Eq. (12).

$$A_{i} = \text{Treatment}_{\text{final}} - \text{Control}_{\text{final}} = R2 - \Delta A + M_{R2} + M_{A},$$
(12)

2.6. Statistical analysis

Regression lines of the model estimates were fitted using the PROC REG function and Pearson's correlation coefficients were obtained using the PROC CORR function of SAS software (SAS Institute, 2001).

3. Results

3.1. Earthworm populations

Only three earthworm species, *A. caliginosa, L. terrestris* and *A. longa*, were found in the enclosures. The naturally-occurring population of *A. longa* earthworms were about 9% of the total

population in 2004 and 2% in 2005. Manipulation of earthworm species was not successful and the proportion of A. caliginosa and L. terrestris did not differ between treatments (Eriksen-Hamel and Whalen, 2007a). This partial failure to manipulate the proportion of earthworm species does not inhibit the use of the "deduction" method as we were able to obtain a wide range of earthworm biomass across all enclosures. The final earthworm biomass of the different treatments ranged from 25 to 92 g fw m^{-2} in 2004 and 23 to 44 g fw m^{-2} in 2005, suggesting greater survival and growth of earthworms introduced to enclosures during 2004 than 2005 (Table 1). Populations from this particular field site were intensively studied in field surveys, manipulation studies and laboratory experiments (Eriksen-Hamel and Whalen, 2007a,b; Perreault et al., 2007; Speratti and Whalen, 2008). As such, we determined that the earthworm populations within the enclosures were well below the levels at which density-dependent growth constraints were observed.

3.2. Secondary production estimates

The relationships between secondary production and earthworm biomass were significant in both years (Fig. 3). When results from this study were extrapolated for a 35 week growing season and combined with other published data from temperate ecosystems, a strong correlation (r = 0.90, p < 0.001) exists between secondary production of Lumbricid earthworms and mean earthworm biomass (Fig. 4). The difference between measured and estimated values of A_i ranged from 0.1 to 6.5 g fw m⁻² for *A. caliginosa* and 16 to 28 g fw m⁻² for *L. terrestris* in 2004, and 0.7 to 17 g fw m⁻² for *A. caliginosa* and 13 to 24 g fw m⁻² for *L. terrestris* in 2005.

4. Discussion

4.1. Secondary production estimates

The estimates of secondary production during the 17 week period determined by the "*deduction*" approach ranged from 3 to



Fig. 3. Relationships between secondary production (*P*) (g AFDW m⁻²) from the "*deduction*" approach and mean earthworm biomass (*B*) (g AFDW m⁻²) during the period June–October in 2004 (\blacksquare) and 2005 (\blacktriangle).



Fig. 4. The relationship between secondary production (*P*) (g AFDW m⁻²) and mean earthworm biomass (*B*) (g AFDW m⁻²) from different studies. Data from this study were extrapolated to a 35 week period, to be consistent with other studies. Pearson's correlation coefficient across all studies is r = 0.90, p < 0.001.

17 g AFDW m⁻² in 2004 and from 2 to 11 g AFDW m⁻² in 2005. If we assume that secondary production during the growing season could be extrapolated for the frost-free period of the year from April to November (35 weeks) and presented on an annual basis, then our estimates $(4-35 \text{ g AFDW m}^{-2} \text{ year}^{-1})$ are similar to the estimates of 4-32 g AFDW m⁻² year⁻¹ reported in other cold temperate arable agroecosystems and calculated using direct methods (Fig. 4) (Boström, 1988; Curry et al., 1995; Whalen and Parmelee, 2000). The only exception is the high secondary production (47 g AFDW m⁻²) recorded in the study of Parmelee and Crossley (1988) which probably arises from the longer frost-free period (February-November) in Georgia, USA than other studies in temperate regions (Ohio (USA), Sweden and Ireland) that have colder winters and a shorter frost-free period. We assumed no cocoon production during this study because peak cocoon production occurs in early spring and late fall (Whalen et al., 1998). The secondary production through cocoons was calculated to be about 4-8% of total tissue production (Parmelee and Crossley, 1988; Curry et al., 1995). Therefore, the secondary production determined by the "deduction" approach could be underestimated by about $0.2-1.7 \text{ g AFDW m}^{-2} \text{ year}^{-1}$.

Secondary production has been measured for the naturallyoccurring earthworm community in forests and agroecosystems of the UK and Ireland (Satchell, 1963; Boström, 1988; Curry et al., 1995), the USA (Parmelee and Crossley, 1988; Whalen and Parmelee, 2000), and in India (Senapati et al., 1991, 1992). Initially, comparison of secondary production estimates between these studies appears to be difficult due to differences in earthworm biomass, species, ecosystem and methods used. However, there is little difference in secondary production estimates for earthworm communities when the size-frequency and IGR methods are used (Whalen and Parmelee, 2000). Therefore, we assume that secondary production estimates from different methods can be compared. Furthermore, none of these studies specifically address the relationship between secondary production and the size of the earthworm community. This can be overcome by presenting secondary production vs mean earthworm biomass (Fig. 4).

The relationship between secondary production (P) and mean biomass (B) indicates the biomass turnover rate of populations, while the reciprocal of the P/B ratio indicates the time required to replace biomass in populations (Benke, 1984). The high correlation

between secondary production and mean earthworm biomass across all studies suggests that population turnover of earthworm communities is related to population size and not necessarily climate or available resources. There was little difference between the average population turnover in 2004 (P/B = 2.3) and 2005 (P/B = 2.0). In a field study in Ohio, the biomass and secondary production of earthworm communities was greater in manure fertilized plots than inorganic fertilized plots, however no significant differences were found between the population turnover in both systems (P/B was 2.5 in manure plots vs 2.2 in inorganic plots) (Whalen and Parmelee, 2000). Similarly, the biomass and secondary production of earthworm communities was greater in a Lucerne field than in a meadow, yet no differences were found between the population turnover in both fields (P/B was 1.4 in Lucerne vs 1.2 in the meadow) (Boström, 1988).

4.2. Limitations and constraints of the "deduction" approach

The "deduction" approach is a reasonable method that can be used if the experimental design prevents repeated sampling or physical disturbance of the experimental plots. The major conditions for using the "deduction" approach are the use of manipulation experiments where individuals are added to a contained area (e.g. laboratory mesocosms or field enclosures), and the inclusion of a control treatment without any added individuals. In addition, accurate estimates of the duration of growth, growth rates, and mortality must be known for the organism in question. The response of earthworm activity to soil temperature and moisture is a more continuous function than the binary (positive or negative activity) system proposed in this method. We believe that this simplified binary system, although not ideal, allows us to make a good estimate of the duration of earthworm activity with a fraction of the computing resources needed to calculate descriptive continuous functions. The IGRs that we used in our study were approximately $0.0008-0.006 \text{ d}^{-1}$ for all species, which are comparable to IGRs calculated in other studies (Mazantseva, 1982; Booth et al., 2000; Wever et al., 2001). Furthermore, we assumed these growth rates were unaffected by population density since earthworm populations in all treatments were lower than the population threshold found to cause significant decreases in growth rates in a laboratory experiment using the same soils and earthworm species (Eriksen-Hamel and Whalen, 2007b). The mortality rates of 35-50% during the 17 week field experiment were based on field and laboratory core experiments (Al-Yousef and Shoreit, 1992; Subler et al., 1997; Boyer et al., 1999; Wever et al., 2001; Eriksen-Hamel and Whalen, 2006, 2007a). We acknowledge that a lack of life-history information for some organisms may limit the widespread use of the "deduction" approach, however it may prove useful to researchers who conduct plot-level manipulation experiments to determine the roles of different species, functional groups and their abundance in terrestrial ecosystems (Blair et al., 1995).

The comparison between the measured and estimated biomass of earthworms added to enclosures (A_i) shows that our estimates of growth and mortality for *A. caliginosa* were more accurate than for *L. terrestris*. The large difference (13–28 g fw m⁻²) for *L. terrestris* is most likely due to overestimation of the survivorship of *L. terrestris* added to enclosures. This suggests that species-specific estimates of growth and mortality would improve the estimates obtained from the "deduction" approach.

A drawback of our experiment was that the earthworm manipulations were done during the summer (June) when soil temperatures were high (>20 °C) and soil moistures were highly variable. Visual observations of casting activity show that earthworms were most active in the early spring (April and May) and autumn (late September and October) when soils were cooler

 $(10-15 \,^{\circ}\text{C})$ and moister. Clearly we have missed peak periods of secondary production and therefore would expect greater biomass production if the experiment had included the entire frost-free period of our temperate agroecosystem. Nonetheless, the extrapolated estimates made by the "*deduction*" approach are within the range of secondary production estimates made by other direct methods (Fig. 4). Yet, this method still requires validation and future field work should compare this approach with other direct methods of assessing secondary production such as the IGR and size-frequency methods.

5. Conclusions

The "deduction" approach to estimating secondary production can be used in studies where frequent and repeated measurement of earthworm biomass is not possible. Researchers require accurate estimates of the duration of earthworm activity, field growth rates and mortality to calculate secondary production with the "deduction" approach and arrive at similar values as those obtained in studies where direct measurements are taken. Further validation of the "deduction" approach, compared to direct methods of calculating earthworm secondary production such as the size-frequency and instantaneous growth rate methods, is recommended. The "deduction" approach can be used broadly to determine secondary production for organisms in small-scale manipulation studies, where repeated removal of individuals or disturbance of the habitats could bias experimental results. This approach increases the set of tools available to ecologists to assess secondary production of organisms and may provide new insights into the energy flow and nutrient fluxes through communities.

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