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Earthworm secondary production and N flux in agroecosystems: a comparison of two approaches

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Abstract Production was estimated for Aporrectodea spp. and Lumbricus spp. populations in corn agroecosystems with a 5-year history of manure or inorganic fertilizer applications during 1994–1995 and 1995–1996. Earthworm biomass and production were greater in manure than inorganic fertilizer plots, although biomass and production declined by about 50% between 1994–1995 and 1995–1996 due to unfavorable climatic conditions. Production was highest during the spring and autumn when soil temperatures were between 4 and 22°C. Production was higher in Lumbricus spp. than Aporrectodea spp. populations due to greater Lumbricus spp. biomass. Aporrectodea spp. production was 3.47-16.14 g ash-free dry weight (AFDW) m⁻² year⁻¹, while *Lumbricus* spp. production was 6.09–18.11 g AFDW m⁻² year⁻¹, depending on the fertilizer treatment and the method used to estimate production. However, production estimates from the instantaneous growth rate method were within 27% of the values calculated using the size-frequency method. Nitrogen flux through earthworms was used to estimate efficiency quotients. Net production efficiency (P/A) ranged from 0.64 to 0.76, assimilation efficiency (A/C) ranged from 0.1 to 0.3, and gross production efficiency (P/C) ranged from 0.06 to 0.22. Annual N flux through earthworm populations was higher in manure than inorganic fertilizer plots, and ranged from 2.95 to 5.47 g N m⁻² year⁻¹ in 1994–1995 and 1.76 to 2.92 g N m⁻² year⁻¹ in 1995–1996. The N flux through earthworms represented an amount equivalent to 16–30% of crop N uptake during 1994–1995 and 11-18% of crop N uptake during 1995-1996. We con-

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Key words Earthworms · Secondary production · Nitrogen · Agroecosystems · Efficiency quotients

Introduction

Ecologists have long sought to explain ecosystem dynamics by examining the flow of energy through plant and animal communities (Lindeman 1942; Odum 1957; Teal 1962). The flow of energy and materials through an animal population can be quantified by determining its secondary production, the accumulation of biomass through growth and reproduction. Secondary production is a comprehensive measure of the success of a population because it combines components of life history, including survivorship, growth rates, development time, and reproduction, with the environmental factors that influence the number and biomass of individuals in a population (Benke and Jacobi 1994). Furthermore, analysis of secondary production can be used to test ecological hypotheses about animal populations. We will demonstrate how secondary production can be used to calculate the N cycled through earthworms in agroecosystems.

It is expected that organic fertilizers will be substituted for inorganic fertilizers to supply nutrients for crop production as we adopt more sustainable farming practices (Edwards 1989; Poincelot 1990). Earthworm populations are often larger in agroecosystems amended with organic fertilizers compared to those receiving inorganic fertilizers (Edwards and Lofty 1982; Werner and Dindal 1989; Tiwari 1993; Whalen et al. 1998), which may result in a greater role of earthworms in soil fertility and crop production. Earthworms are considered keystone species (Parmelee et al. 1998) due to their multiple effects on soil physical, chemical, and biological properties, and are known to regulate decomposition and nutrient cycling processes through their interactions with microorganisms (Blair et al. 1995; Bohlen et al. 1997). They also influence nutrient cycling directly through the consumption of organic materials, assimilation of nutrients into their tissues, and release of nutrients from their biomass through mucus secretion, urine excretion and mortality (decomposition). Earthworms process an estimated 2-15 Mg ha⁻¹ year⁻¹ of organic matter from soil organic matter and litter (Satchell 1967; Shipitalo et al. 1988; Lavelle et al. 1989; Hendriksen 1991; James 1991; Whalen and Parmelee 1999a), and the annual flux of N through earthworm biomass in temperate, cultivated agroecosystems has been estimated to range from 10 to 74 kg N ha⁻¹ year⁻¹ (Andersen 1983; Christensen 1987; Böström 1988; Parmelee and Crossley 1988; Curry et al. 1995). Nitrogen released from earthworms can be significant relative to crop N requirements, and Parmelee and Crossley (1988) noted that the flux of 63 kg N ha⁻¹ year⁻¹ through earthworms in a no-till agroecosystem represented an amount equivalent to 38% of the total N uptake by the sorghum crop. However, we are not aware of any studies that have compared N flux through earthworms in cultivated agroecosystems receiving manure or inorganic fertilizer amendments.

Accurate estimates of earthworm secondary production are required to determine the N flux through earthworm populations using the methods outlined in this study. Field estimates of earthworm secondary production are 2.9–8.7 g of tissue (dry weight) m⁻² year⁻¹ for A. caliginosa in cultivated barley and ley plots in Sweden (Böström 1988), 5.2 and 11.4 g m⁻² year⁻¹ for *Drawida* willsi Michaelsen in a rice field in India (Senapati et al. 1991), and 44.3 g m⁻² year⁻¹ for earthworm populations dominated by Lumbricus rubellus and Aporrectodea turgida in a no-tillage agroecosystem in Georgia, United States (Parmelee and Crossley 1988). The secondary production of the earthworm populations in a winter cereal agroecosystem in Ireland dominated by Allolobophora chlorotica and Aporrectodea caliginosa ranged from 16.3 to 43.7 g m⁻² year⁻¹ over a 3-year period (Curry et al. 1995). However, most of these estimates are made using methods that follow growth of distinct cohorts during a period of time. Earthworm populations are not synchronous, and populations contain adults and juveniles in different stages of development. In this situation, Benke (1993) suggests that secondary production will be calculated most accurately using either the instantaneous growth rate method or the size frequency method.

The objectives of this study were (1) to compare the instantaneous growth rate and size frequency methods of calculating secondary production for earthworm populations, (2) to determine the secondary production of earthworms in corn agroecosystems with a 5-year history of manure or inorganic fertilizer amendments, and (3) to estimate the annual N flux through earthworms in corn agroecosystems with a 5-year history of manure or inorganic fertilizer amendments.

Materials and methods

Study site

The study was conducted in established corn agroecosystems at the Ohio Agricultural Research and Development Center in Wooster, Ohio, United States. Mean monthly temperatures ranged from -4.8° C in January to 21.2° C in July and the mean annual precipitation was 1010 mm. Meterological data were obtained from the Ohio Agricultural Research and Development Center weather station which reports daily mean air and soil temperatures, precipitation, evapotranspiration, wind speed, and solar radiation.

The experimental site was a relatively flat area on a fine, mixed, mesic Fragiudalf of the Canfield series (Luvisol), a major agricultural soil type in the region. The soil texture was silt loam (135 g sand kg⁻¹, 737 g silt kg⁻¹, 128 g clay kg⁻¹) with a cation exchange capacity of 10 cmol_c kg⁻¹. The pH of this soil was 6.3 and it contained 23 g organic C kg⁻¹ and 1.9 g N kg⁻¹. The site was planted with corn (*Zea mays*) from 1984 to 1987 and with alfalfa (*Medicago sativa*) from 1987 to 1991. However, since 1992; the site has been used for continuous corn production under a conventional disk tillage system.

Experimental design

In the spring of 1991, 12 field plots $(20\times30 \text{ m})$ were laid out in a randomized complete block design with four replicates of each of three agroecosystem nutrient treatments: (1) NH₄NO₃ fertilizer (inorganic), (2) legume/rye (*Vicia villosa/Secale cereale*) cover crop (legume), and (3) straw-pack dairy cow manure (manure). The plots were planted with soybeans in the spring of 1991 and no nutrients were applied. Beginning in 1992, amendments were applied in spring at a rate of approximately 150 kg N ha⁻¹ year⁻¹ and disk-incorporated to 10 cm depth in late May or early June prior to seeding. Results from the legume/rye treatment are not presented. The herbicides cyanazinde, alachlor, and paraquat were used for weed control. Corn residues were left on the plots after harvest and incorporated by disking the following spring. Further information about this site has been reported in Bohlen et al. (1997).

Earthworm sampling

The earthworm species found at the site included *Aporrectodea tuberculata* Eisen, *A. trapezoides* Dugés, *Lumbricus terrestris* L. and *L. rubellus* Hoffmeister. Earthworm populations were sampled in the manure and inorganic fertilizer plots at approximately monthly intervals during the spring/early summer and autumn, when temperature and moisture conditions were most conducive to earthworm growth. In the autumn of 1994, earthworm populations were sampled on 29 September, 3 November, and 6 December. In 1995, earthworm populations were sampled on 29 September, 3 November, and 6 December. In 1995, earthworm populations were sampled on 20 April, 16 May, 16 June, 13 July, 10 October, and 27 November, and in 1996, hey were sampled on 18 April, 14 May, 20 June, 18 September, and 24 October. The 1994–1995 results include earthworms collected from 29 September 1994 to 10 October 1995 and the 1995–1996 results include earthworms collected from 27 November 1995 to 24 October 1996.

Earthworms were collected from four randomly selected locations in each manure- and inorganic fertilizer-amended plots first by hand-sorting 38×38 cm quadrats to a depth of 15 cm. Dilute formalin (0.25%) was then poured onto the bottom of each quadrat to collect the deeper-dwelling *L. terrestris*. Between 90 and 95% of earthworms in quadrats were collected by combined handsorting and formalin extraction (Blair et al. 1996), and earthworms were stored in a 5% formalin solution until species identification could be made. On each sampling date, two 10.5 cm (diameter)×15 cm (deep) cores were taken adjacent to three of the four earthworm quadrats in each plot to assess the number and biomass of cocoons. Soil from cores was wet-sieved through stacked screens (top screen mesh size=2.38 mm, bottom screen mesh size=0.79 mm) to collect earthworm cocoons.

Earthworms were separated into age classes on the basis of clitellum development, and were categorized as fragments (incomplete earthworm fragments), juveniles, pre-clitellate adults (clitellum present but not fully-developed) and clitellate adults (fullydeveloped clitellum). Sexually mature specimens were identified to the species level using the key by Schwert (1990). The dominant earthworm species in both manure and inorganic fertilizer plots, based on mature (adult and pre-clitellate) earthworms, were *A. tuberculata* and *L. terrestris*, and the majority of juveniles were assumed to belong to these species (Whalen et al. 1998). It was not possible to assign cocoons to earthworm species because *A. tuberculata*, *A. trapezoides*, and *L. rubellus* produce cocoons of similar shape and size. Cocoons deposited by *L. terrestris* are identified easily because they are twice as large as cocoons produced by the other species.

The number of individuals was censused by a head count of intact specimens and fragments that contained an identifiable head, and the body length of each intact specimen was measured. Earthworms and cocoons were oven-dried (60° C for 48 h) and then ashed (500° C for 4 h) to determine biomass on an ash-free dry weight (AFDW) basis. The number and biomass of individuals of *A. trapezoides* and *L. rubellus* were combined with the dominant species and results are presented for *Aporrectodea* spp. and *Lumbricus* spp. populations.

Relationship between earthworm length and biomass

Earthworms do not pass through distinctive stages as they develop and hence cohorts cannot be identified readily. The size-frequency method of estimating secondary production requires individuals to be categorized in "cohorts", which we defined from body length measurements. Earthworm length data was separated into eight classes, or "cohorts" of 2-cm increments (0-1.9 cm, 2-3.9 cm, 4-5.9 cm, 6-7.9 cm, 8-9.9 cm, 10-11.9 cm, 12-13.9 cm, and >14 cm). Individuals of Aporrectodea spp. were generally sexually mature once they had reached a body length of 8-10 cm, and seldom exceeded 12 cm. Sexual maturity for L. terrestris occurred when individuals achieved a body length of 12-14 cm, and adults can grow as long as 18 cm. The growth rates of Aporrectodea spp. and Lumbricus spp. tend to decline as earthworm mass increases (Andersen 1987; Whalen and Parmelee 1999b), so the calculation of a size-specific instantaneous growth rate (IGR) for earthworm "cohort" should reduce the variability in estimated growth of individuals in different stages of development.

Biomass measurements at each sampling date were for groups of individuals (adults, juveniles), while length measurements were made for each individual collected. We determined the mean biomass of individuals in each length class by collecting a random sample of earthworms (n=325), ranging in age from small juveniles to adults, from an area adjacent to the study site in May 1997. Earthworms were expelled from soil by pouring dilute (0.25%) formalin onto a mowed area, collected and stored in 5% formalin solution. Individuals were identified, lengths measured, oven-dried (60° C for 48 h) and ashed (500° C for 4 h) to determine ash-free dry weight (AFDW). The relationship between biomass and body length was:

$$lnAFDW = lna + b \times lnBL$$
(1)

where AFDW was individual mass (ash-free dry weight, g), BL was body length (cm), *a* was a constant, and *b* was the slope of the regression equation. The relationship between biomass and body length for *Aporrectodea* spp. was:

AFDW=0.0016×BL^{1.9262} (r^2 =0.82, n=110, P<0.0001) (2)

while the relationship for Lumbricus spp. was:

AFDW=0.000517×BL^{2.6656} (
$$r^2$$
=0.94, n =215, P <0.0001) (3)

Earthworm biomass at each sampling date was calculated by summing the product of the mean number of earthworms (number m^{-2}) by the mean ash-free dry weight for each length class where:

$$B = \sum \{ N \times [AFDW_{i\times}(n_i/n_T)] \}$$
(4)

and B is biomass, N is the mean number of earthworms (number m^{-2}), AFDW_j is the mean ash-free dry weight of the earthworms in a length class, n_j is the number of earthworms in the jth length class (*j*=1 to 8) and n_T is the total number of earthworms measured at a given sampling date.

Secondary production using the instantaneous growth rate (IGR) method

The IGR method combines a daily IGR from laboratory or field growth trials with field biomass measurements (Benke 1984). Growth of juveniles of *Aporrectodea* spp. and *Lumbricus* spp. was measured in manure and inorganic-fertilizer plots during time intervals that corresponded generally to biomass sampling dates during the spring and autumn of 1995 and 1996. Details of this study and calculation of the IGR for earthworms have been described by Whalen and Parmelee (1999b).

Earthworm growth under field conditions was measured successfully in the study described by Whalen and Parmelee (1999b), but we did not have growth measurements for earthworms in all length classes during some growth intervals. It is not appropriate to use a single growth rate for earthworms of varying ages because the IGR is affected by earthworm size (Whalen and Parmelee 1999b). There is a linear relationship between earthworm initial (y_t) and final mass (Y_T) , and regression equations relating initial and final mass of Aporrectodea spp. and Lumbricus spp. under field conditions are given in Table 1. Regression equations were used predict the final mass (Y_T) of earthworms in each length class during growth intervals (T-t) in the field, and size-specific IGRs for each length class were calculated using equations given in Whalen and Parmelee (1999b). Sample calculations of size-specific IGRs for Aporrectodea spp. and Lumbricus spp. in manureamended agroecosystems from 29 September to 3 November 1994 are provided in Table 2.

Earthworm IGRs are used to calculate growth during the period between biomass sampling dates, but we did not grow earthworms in the field during all periods between biomass sampling. Whalen and Parmelee (1999b) found in laboratory studies that the IGR of A. tuberculata was affected more by soil temperature than soil moisture, and we assumed that mean soil temperatures during the periods between biomass sampling would reflect earthworm growth under field conditions. The regression equations from Table 1 were used to estimate earthworm growth for periods between biomass sampling with similar mean soil temperatures $(\pm 2^{\circ}C)$. We assumed earthworms did not grow when soil temperatures fell below 4°C or exceeded 22°C because we did not observe earthworm activity under these conditions (Whalen et al. 1998; Whalen and Parmelee 1999b). It is well documented that earthworm populations are influenced significantly by soil temperature, and in temperate agroecosystems, earthworms are usually most active during spring and autumn (Curry et al. 1995; Parmelee and Crossley 1988). Endogeic earthworms like Aporrectodea spp. survive unfavorable environmental conditions by aestivating, while anecic earthworms like Lumbricus spp. retreat to the bottom of deep vertical burrows and may enter a quiescent state.

Size-specific estimates of earthworm biomass were calculated and combined with size-specific growth rates to estimate secondary production using the IGR method (Benke 1984) as:

$$P = \sum \{ \text{IGR}_i \times [(B_{fi} + B_{ii})/2] \times t \}$$
(5)

where *P* is production (g AFDW m⁻²), IGR_{*j*} is the instantaneous growth rate of individuals in the jth length class (*j*=1 to 8), and B_{jj} and B_{ij} are the final and initial standing stock biomasses of individuals in the jth length class observed over a time interval, t,

Table 1 The relationship between earthworm initial weight (y_i) and final weight (Y_T) under different conditions of soil temperature in manure and inorganic fertilizer amended corn agroecosys-

tems for *Aporrectodea* spp. and *Lumbricus* spp. Data are from Whalen and Parmelee (1999b)

| Species | Year | Date | Mean soil | Manure | | | Inorganic fertilizer | | |
|--------------|------|--------|-----------|-----------------------------------|----|-------|------------------------------------|----|-------|
| | | | (°C) | Regression equation | п | r^2 | Regression equation | п | r^2 |
| Aporrectodea | 1995 | 13 Apr | 9.7 | $Y_{\tau}=0.0399+1.1657v_{t}$ | 36 | 0.97 | $Y_{\tau}=0.0330+1.1345v_{t}$ | 39 | 0.90 |
| Aporrectodea | 1995 | 31 May | 20.2 | $Y_{T} = 0.0642 + 0.8367 v_{t}$ | 35 | 0.94 | $Y_{\tau} = 0.0465 + 0.9052 y_{t}$ | 39 | 0.95 |
| Aporrectodea | 1995 | 2 Nov | 3.4 | $Y_{T} = 0.0187 + 1.0416 y_{t}$ | 23 | 0.97 | $Y_{\tau} = 0.0582 + 0.9286 y_{t}$ | 19 | 0.86 |
| Aporrectodea | 1996 | 17 Apr | 13.0 | $Y_{T} = 0.0818 + 1.0092 y_{t}$ | 24 | 0.89 | $Y_{\tau} = 0.0633 + 1.1235 y_{t}$ | 24 | 0.86 |
| Aporrectodea | 1996 | 25 May | 18.4 | $Y_{T} = 0.0871 + 1.0500 y_{t}$ | 26 | 0.92 | $Y_{T} = 0.0466 + 1.0397 y_{t}$ | 23 | 0.94 |
| Aporrectodea | 1996 | 8 Oct | 9.7 | $Y_T = 0.0481 + 1.2553y_t$ | 16 | 0.94 | $Y_T = 0.0498 + 1.2220 y_t$ | 18 | 0.94 |
| Lumbricus | 1995 | 13 Apr | 9.7 | $Y_{\tau} = 0.0571 + 1.0270y_{t}$ | 23 | 0.96 | $Y_{\tau} = 0.1961 + 1.0086y_{t}$ | 23 | 0.98 |
| Lumbricus | 1995 | 31 May | 20.2 | $Y_{T} = 0.3241 + 0.8076y_{t}$ | 13 | 0.94 | $Y_{\tau} = 0.0724 + 1.0195 y_{t}$ | 20 | 0.93 |
| Lumbricus | 1995 | 2 Nov | 3.4 | $Y_{T} = 0.1520 + 0.9303y_{t}$ | 15 | 0.96 | $Y_{\tau} = 0.1422 + 0.9260 y_{t}$ | 17 | 0.98 |
| Lumbricus | 1996 | 17 Apr | 13.0 | $Y_{T} = 0.0870 + 1.0980 y_{t}$ | 25 | 0.96 | $Y_{T} = 0.0667 + 0.9472 y_{t}$ | 23 | 0.85 |
| Lumbricus | 1996 | 25 May | 18.4 | $Y_{T} = 0.0634 + 0.9807 y_{t}$ | 17 | 0.98 | $Y_{\tau} = 0.0190 + 0.9664 y_{t}$ | 15 | 0.97 |
| Lumbricus | 1996 | 8 Oct | 9.7 | $Y_T = 0.1441 + 1.0977 y_t$ | 5 | 0.99 | $Y_T = 0.1550 + 0.9632 y_t$ | 6 | 0.94 |

Table 2 Calculation of size-specific instantaneous growth rates (IGR) for *Aporrectodea* spp. and *Lumbricus* spp. in manureamended plots from 29 September to 3 November 1994 (time=35 days) using data from Whalen and Parmelee (1999b). Mean soil temperature was 13.4° C (*AFD*=ash-free dry weight)

| Length class (cm) | Mean initial biomass (y_t) (g AFDW) | Predicted final biomass (Y_T) (g AFDW) | Estimated IGR(days ⁻¹) |
|----------------------|---------------------------------------|--|---------------------------------------|
| Aporrectodea s | pp. | | |
| 0-1.9 | 0.0027 | 0.0845 | 0.0983 |
| 2-3.9 | 0.0087 | 0.0906 | 0.0669 |
| 4-5.9 | 0.0215 | 0.1035 | 0.0449 |
| 6-7.9 | 0.0400 | 0.1222 | 0.0319 |
| 8–9.9 | 0.0669 | 0.1494 | 0.0229 |
| 10-11.9 | 0.0914 | 0.1740 | 0.0184 |
| 12-13.9 | 0.1313 | 0.2143 | 0.0140 |
| >14 | 0 | 0 | 0 |
| Lumbricus spp. | | | |
| 0-1.9 | 0.0023 | 0.0895 | 0.1046 |
| 2-3.9 | 0.0104 | 0.0984 | 0.0642 |
| 4-5.9 | 0.0366 | 0.1272 | 0.0356 |
| 6–7.9 | 0.1059 | 0.2033 | 0.0186 |
| 8–9.9 | 0.2039 | 0.3108 | 0.0121 |
| 10-11.9 | 0.3203 | 0.4387 | 0.0090 |
| 12-13.9 | 0.4744 | 0.6079 | 0.0071 |
| >14 | 0.8434 | 1.0130 | 0.0052 |

measured in days. Annual earthworm production was estimated by adding cocoon production to P from Eq. 5 for *Aporrectodea* spp. and *Lumbricus* spp. We assumed individuals were recruited into the population from cocoons deposited in June 1995 and June 1996 because maximum annual cocoon production occurred at these sampling dates (Whalen et al. 1998).

Secondary production using the size-frequency (SF) method

The size-frequency method assumes the mean size-frequency distribution during a year is equivalent to an "average cohort" (Hynes and Coleman 1968; Hamilton 1969; Benke 1979). The production of the annual cohort equals the sum of production losses between two length classes multiplied by the total number of length classes. The biomass lost between length classes was calculated by multiplying the decrease in density (ΔN_c) from one length class to the next by the mean mass of individuals between classes (\overline{W}_c). Annual production was calculated by multiplying cohort production by the cohort production interval (CPI) (Benke 1979) where:

$$P = \{ \sum [(\Delta N_c \times \overline{W}_c) \times \mathbf{h}] \} \times CPI$$
(6)

P is production (g AFDW m⁻²), N_c is the decrease in the number of individuals from two consecutive length classes, \overline{W}_c is the mean mass of individuals from two consecutive length classes, h is the number of length classes, and CPI is the cohort production interval, which is the time it takes for a newly hatched individual to achieve its final size. The time to maturity for an individual *A. tuberculata* in the laboratory was estimated to be 300 days (Andersen 1987), while the time to maturity for *L. terrestris* in a forest ecosystem was estimated to be 2 years (Lakhani and Satchell 1970). We assumed a CPI of 1 for *Aporrectodea* spp. (CPI=365 days/365 days) and 0.5 for *Lumbricus* spp. (CPI=365 days/730 days). Cocoon production is not required in the SF method of calculating production because the CPI accounts for recruitment of new generations in a population.

Nitrogen flux through *Aporrectodea* spp. and *Lumbricus* spp. populations

The annual flux of nitrogen through earthworm biomass was calculated by multiplying the percentage nitrogen content of earthworms by the annual earthworm production and adding nitrogen lost through metabolic processes:

N flux=
$$(P \times N_{\text{percent}}) + E$$
 (7)

where N flux is the flow of nitrogen through earthworms expressed as g N m⁻² year⁻¹, *P* is production (g AFDW m⁻²), N_{percent} is the N content of earthworm tissue (g N kg⁻¹ AFDW), and *E* is nitrogen released from earthworm tissues via mucus secretion and urine excretion. It is assumed that annual mortality (g AFDW year⁻¹) is equal to annual production (Andersen 1983), which is supported by field studies that indicate that annual earthworm mortality is approximately equivalent to annual earthworm production (Böström 1988; Christensen 1988). The tissue of dissected juveniles of *A. tuberculata* and *L. terrestris* was oven-dried and ground, and the N content was determined using a Carlo-Erba CN analyzer. The N contents of *Aporrectodea* spp. and *Lumbricus* spp. tissues were 124.2±0.9 g N kg⁻¹ AFDW and 121.4±2.2 g N kg⁻¹ AFDW, respectively.

The daily N loss from *A. tuberculata* and *L. terrestris* from mucus secretion and urine excretion was measured in a laboratory study described by Whalen et al. (2000). Earthworms were labeled with ¹⁵N by providing earthworms with ¹⁵N-labeled organic sub-

strates for 5–6 weeks and measuring the quantity of ¹⁵N excreted in unlabeled soil was measured after 48 h. The mean (±SE) daily N excretion rates for *A. tuberculata* and *L. terrestris* juveniles were 576±25 µg N g⁻¹ earthworm (wet weight) day⁻¹ and 301±16 µg N g⁻¹ day⁻¹, respectively (Whalen et al. 2000).

Earthworm excretion rates were converted from units of $\mu g~N~g^{-1}$ earthworm (wet weight) day-1 to units of $\mu g~N~g^{-1}$ AFDW day-1 for consistency with secondary production measurements. A random sample (n=77) of earthworms ranging in age from small juveniles to adults was collected from an area adjacent to the study site by hand sorting and formalin extraction in April 1995. The earthworms were placed on wet filter paper for 24 h to void their guts, weighed individually (wet weight), killed by immersion in 5% formalin solution, oven-dried (60°C for 48 h) and ashed (500°C for 4 h). AFDW averaged 14% of earthworm wet weight, and the mean N excretion rates for A. tuberculata and L. terrestris juveniles were 1.36 mg N g⁻¹ AFDW day⁻¹ and 0.71 mg N g⁻¹ AFDW day⁻¹, respectively. We assumed these rates represented N losses from active earthworms since it is likely that N losses from aestivating Aporrectodea spp. or quiescent Lumbricus spp. are negligible. The N excretion rate of A. tuberculata and L. terrestris juveniles were applied to all individuals of Aporrectodea spp. and Lumbricus spp. collected in this study because there were many more juvenile than adult earthworms in these populations at all sampling dates (Whalen et al. 1998). Annual N excreted was estimated by multiplying annual mean biomass (g AFDW m⁻²) by the mean nitrogen excretion rate (mg N g⁻¹ AFDW day⁻¹) and the number of days earthworms were active. Earthworms were assumed to be active when mean soil temperatures were between 4°C and 22°C, which was 125 days in 1994–1995 and 137 days in 1995-1996.

We then corrected our estimate of E in Eq. 7 because we believe our method could overestimate N losses from earthworms. Earthworm tissue N is partitioned into structural N (in e.g., muscle, cutin, reproductive structures) and labile N (in e.g., mucusproducing cells in the intestinal tract and on the body surface, coelemic fluid). There is evidence from ¹⁵N-labeling studies that N turnover from the labile N pool is much more rapid than from the structural N pool (Bouché 1984; Barois et al. 1987; Curry et al. 1995), but the size of structural and labile N pools in earthworms has not yet been determined. The equation we used to calculate N excreted by earthworms assumes that N is partitioned equally between structural and labile N pools. If 75% of earthworm N was in the structural N pool and 25% was in the labile N pool, and the pools were uniformly labeled with ¹⁵N, then total N excreted would be 50% less than we have calculated (Whalen et al. 2000).

The use of stable isotopes to calculate nutrient turnover by earthworms has been questioned (e.g., Schmidt et al. 1999) because earthworms may not incorporate tracers such as ¹⁵N into tissues uniformly unless the labeling period is sufficiently long. We are not aware of published studies that have determined the length of time required to uniformly label earthworms with ¹⁵N. The assumption of uniform labeling of earthworms with ¹⁵N does not overestimate total N excretion as much as the assumption of equal pool sizes of

structural and labile N. If the structural N and labile N pools contain 75% and 25%, respectively, of earthworm N, and earthworms are not uniformly labeled with ¹⁵N (25% of ¹⁵N in structural N pool and 75% of ¹⁵N in labile N pool), then total N excreted would be 66% less than calculated (Whalen et al. 2000). There is no information available in the literature currently that would allow us to correct our data for non-uniform labeling in earthworm structural and labile N pools. The value of E in Eq. 7 was reduced by 66% so that our estimate of N flux from earthworms through mucus secretion and urine excretion would be conservative.

Results

Secondary production of *Aporrectodea* spp. and *Lumbricus* spp. using the IGR method

A sample calculation of *Aporrectodea* spp. production in manure-amended plots during 1994–1995 using the IGR method is presented in Tables 3 and 4. Production of *Aporrectodea* spp. was between 3.47 and 16.14 g m⁻² year⁻¹ during the study (Table 5). *Aporrectodea* spp. production was lower in inorganic fertilizer than manure plots during both years and there was a decline in *Aporrectodea* spp. numbers and biomass in all plots between 1994–1995 and 1995–1996 (Table 5). Production of *Aporrectodea* spp. in manure and inorganic fertilizer plots was greatest from April to June of 1995 and 1996 (Fig. 1A), and the annual *P/B* ratio for *Aporrectodea* spp. ranged from 3.2 to 4.6 (Table 5).

Production of *Lumbricus* spp. was lower in inorganic fertilizer than manure plots in both years, and ranged from 6.09 to 16.95 g m⁻² year⁻¹ (Table 5). The number of Lumbricus spp. declined by 48% in manure plots and 24% in inorganic fertilizer plots between 1994–1995 and 1995–1996; and the annual P/B ratio was between 1.4 and 2.4 (Table 5). The production of *Lumbricus* spp. was greatest in the spring of 1995 (Fig. 1B). Aporrectodea spp. and Lumbricus spp. production declined after June of each year, and increased as conditions became more favorable to earthworm growth during September or October of each year (Fig. 1A,B). Cocoon production tended to be greater in manure-amended than inorganic fertilizer plots, and annual production of earthworm communities in corn agroecosystems ranged from 16.86 to 33.16 g m⁻² year⁻¹ in 1994–1995 and 9.6 to 16.43 g m⁻² year⁻¹ in 1995–1996 (Table 5).

Table 3 Instantaneousgrowth rates for Aporrectodea

spp. in each length class (IGR_{*i*}) in 1994–1995 calculated from temperature-dependent growth rate equations (Table 1) and mean soil temperatures (*T*) between sampling dates. Growth was assumed to be zero when mean soil temperature fell below 4°C or exceeded 22°C

| Date | $T(^{\circ}C)$ | IGR_j (day ⁻¹) | | | | | | | | | |
|---|--|---|--|--|--|---|---|--------------------------------------|---------------------------------|--|--|
| | | Length c | Length class (j) (cm) | | | | | | | | |
| | | 0–1.9 | 2-3.9 | 4–5.9 | 6–7.9 | 8–9.9 | 10–11.9 | 12-13.9 | >14 | | |
| 29 Sept 3 Nov 6 Dec 20 Apr 16 May 16 June 13 July | 13.4 7.4 3.2 11.4 18.0 22.9 22.0 | $\begin{array}{c} 0.0983 \\ 0.0879 \\ 0 \\ 0.1184 \\ 0.0844 \\ 0 \\ 0 \\ \end{array}$ | $\begin{array}{c} 0.0669\\ 0.0521\\ 0\\ 0.0587\\ 0.0543\\ 0\\ 0\\ \end{array}$ | $\begin{array}{c} 0.0449\\ 0.0337\\ 0\\ 0.0402\\ 0.0315\\ 0\\ 0\\ \end{array}$ | $\begin{array}{c} 0.0319\\ 0.0228\\ 0\\ 0.0263\\ 0.0193\\ 0\\ 0\\ \end{array}$ | 0.0229 0.0181 0 0.0195 0.0119 0 0 | 0.0184 0.0153 0 0.0159 0.0076 0 0 | 0.0140 0 0.0127 0 0 0 | 0 0 0 0 0 0 0 | | |

Table 4 Secondary production of *Aporrectodea* spp. in manure-amended plots of corn agroecosystems during 1994–1995 calculated by the instantaneous growth rate (IGR) method, using data from Table 3. Mean biomass for earthworms in each length class (B_i) , production (P_i) during time intervals between sampling dates (t), and estimated annual production (P)

| Date | B_j (g AFDW m ⁻²) | | | | | | | | | | |
|---------|---------------------------------|--------|--------|--------|--------|---------|---------|-----|----------|-------|--|
| | Length class (<i>j</i>) (cm) | | | | | | | | | | |
| | 0–1.9 | 2–3.9 | 4–5.9 | 6–7.9 | 8–9.9 | 10–11.9 | 12–13.9 | >14 | t (days) | P_t | |
| 29 Sept | 0.0455 | 0.9987 | 1.3734 | 1.5829 | 1.2907 | 0.3162 | 0.1298 | 0 | 35 | 6.20 | |
| 3 Nov | 0.0094 | 0.4810 | 0.7164 | 1.1841 | 1.0536 | 0.3405 | 0 | 0 | 33 | 2.90 | |
| 6 Dec | 0 | 0.2466 | 0.4556 | 1.0320 | 0.9728 | 0.3322 | 0 | 0 | 135 | 0 | |
| 20 Apr | 0.0019 | 0.1760 | 0.5343 | 0.9106 | 1.2602 | 0.6209 | 0.0858 | 0 | 26 | 3.85 | |
| 16 May | 0.0053 | 0.4360 | 1.3558 | 2.9215 | 1.9047 | 0.6364 | 0 | 0 | 31 | 3.19 | |
| 16 June | 0.0036 | 0.1147 | 0.4675 | 0.8145 | 1.1208 | 0.5852 | 0 | 0 | 27 | 0 | |
| 13 July | 0.0076 | 0.1315 | 0.1799 | 0.2165 | 0.2452 | 0.0879 | 0 | 0 | 89 | 0 | |
| 10 Oct | 0.0164 | 0.0694 | 0.1297 | 0.3625 | 0.0537 | 0.0623 | 0 | 0 | | | |

Table 5 Summary of the annual mean abundance, mean biomass, production and the *P/B* ratio for *Aporrectodea* spp. and *Lumbricus* spp. in corn agroecosystems receiving manure or inorganic fertilizer amendments using the IGR method to estimate secondary production

| Year | Species | Density (no. m ⁻²) | Biomass (g AFDW m ⁻²) | Total production (g AFDW m ⁻² year ⁻¹) | <i>P/B</i> ratio |
|-------------------------------------|--|-----------------------------------|--------------------------------------|--|------------------|
| Manure | | | | | |
| 1994–1995 1994–1995 1994–1995 | <i>Aporrectodea</i> spp. <i>Lumbricus</i> spp. Cocoons | 99.5 87.8 7.1 | 3.51 9.14 0.029 | 16.14 16.95 0.067 | 4.6 1.9 |
| | Total | 194.4 | 12.68 | 33.16 | 2.6 |
| 1995–1996 1995–1996 1995–1996 | <i>Aporrectodea</i> spp. <i>Lumbricus</i> spp. Cocoons | 33.8 45.9 3.5 | 1.52 4.80 0.016 | 4.92 11.44 0.072 | 3.2 2.4 |
| | Total | 83.2 | 6.34 | 16.43 | 2.6 |
| Inorganic fert | iliser | | | | |
| 1994–1995 1994–1995 1994–1995 | <i>Aporrectodea</i> spp. <i>Lumbricus</i> spp. Cocoons | 53.4 51.0 5.2 | 2.22 5.62 0.021 | 7.79 9.02 0.053 | 3.5 1.6 |
| | Total | 109.6 | 7.86 | 16.86 | 2.1 |
| 1995–1996 1995–1996 1995–1996 | Aporrectodea spp. Lumbricus spp. Cocoons Total | 26.9 38.9 2.3 | 0.92 4.27 0.009 5 20 | 3.47 6.09 0.043 9.60 | 3.8 1.4 |

Life history of *Aporrectodea* spp. and *Lumbricus* spp. populations

There were significantly more individuals of *Aporrect-odea* spp. and *Lumbricus* spp. in manure than inorganic fertilizer plots, however the proportion of individuals in each length class was not affected significantly by fertilizer amendments (Whalen et al. 1998). The size-frequency diagrams for *Aporrectodea* spp. and *Lumbricus* spp. in manure-amended and inorganically-fertilized plots indicate that the life history of both populations was asynchronous and cohorts were not identified readily (Figs. 2, 3, 4, 5).

In the first year of the study, the greatest numbers of large individuals (>8 cm) of *Aporrectodea* spp. were collected in June 1995 from manure plots (30.3% of individuals) and in December 1994 and June 1995 in inorganic fertilizer plots (30.5% and 30.2% of individuals) (Figs. 2, 3). In 1995–1996, the greatest numbers of large

earthworms (>8 cm) of *Aporrectodea* spp. were found in May and June of 1996 in manure (33.8% and 52% of individuals) and inorganic fertilizer plots (24.3% and 25.8% of individuals) (Figs. 2, 3).

The proportion of large earthworms (>10 cm) of *Lumbricus* spp. was greatest in manure plots in June and July 1995 (36.8% and 50% of individuals) and June 1996 (42.9% of individuals) (Fig. 4). In the inorganic fertilizer plots, the largest numbers of *Lumbricus* spp. in length classes greater than 10 cm were collected in July 1995 (47.4% of individuals) and June 1996 (27.3% of individuals) (Fig. 5). The larger proportion of individuals of *Aporrectodea* spp. and *Lumbricus* spp. populations in the largest length classes during December 1994, June and July 1995, and May and June 1996, corresponded to significantly greater numbers of cocoons collected at these dates compared to other sampling dates (Whalen et al. 1998).

A pulse of individuals in the smallest length classes (0-1.9 cm and 2-3.9 cm), relative to the total number of

individuals, of *Aporrectodea* spp. was observed in the manure plots in October 1994 (50.9% of individuals), July and October 1995 (51.9% and 50% of individuals), and April and October 1996 (42.9% and 39.7% of individuals) (Fig. 2). In inorganic fertilizer plots, the greatest number of small (<4 cm) *Aporrectodea* spp. were collected in October 1994 (29.8% of individuals), July and



Fig. 1 Production dynamics of **A** *Aporrectodea* spp. and **B** *Lumbricus* spp. in manure and inorganic fertilizer plots during 1994–1996. Production [g ash-free dry weight (AFDW) m⁻²] dynamics are expressed as cumulative secondary production between biomass sampling dates

Fig. 2 Size frequency distribution of *Aporrectodea* spp. in manure plots during 1994–1996. *Width of horizontal bars* indicates the relative frequency of occurrence of *Aporrectodea* spp. in each length class. *Numbers above histograms* indicate the number of earthworms measured at each date October 1995 (68.9% and 47.5% of individuals), and April and September of 1996 (47.6% and 48% of individuals) (Fig. 3).

The greatest proportions of small (<4 cm) *Lumbricus* spp. were found in October 1994, October 1995, and September 1996 in both manure (62%, 84.1% and 55.9% of individuals) and inorganic fertilizer (70.8%, 84.2%, and 65.4% of individuals) plots (Figs. 4, 5). The size-frequency histograms suggest that cohorts of *Aporrectodea* spp. and *Lumbricus* spp. recruited during autumn finished their life cycle with reproduction during June of the following year. However, separation of cohorts of *Aporrectodea* spp. and *Lumbricus* spp. was difficult since individuals in all length classes and cocoons were found at nearly all sampling dates.

Secondary production of *Aporrectodea* spp. and *Lumbricus* spp. using the SF method

A sample calculation of *Aporrectodea* spp. production in manure-amended plots during 1994–1995 using the SF method is presented in Table 6. Very few individuals were found in the smallest length class (0–1.9 cm), which may have been due to inefficient sampling of the smallest earthworms or rapid growth through this class, and we added these individuals to the next largest length class (2–3.9 cm). The mean number of individuals of *Aporrectodea* spp. in the 0–3.9 cm length class was 37 m⁻² and there were fewer than 1 individual m⁻² in the 12–13.9 cm length class (Table 6). Cohort production for *Aporrectodea* spp. was calculated using six length classes (*h*=6, Eq. 6), and cohort production for the larger *Lumbricus* spp. was calculated using seven length classes.

Production of *Aporrectodea* spp. was higher in the manure plots than in the inorganic fertilizer plots during both years, and ranged from 7.11 to 14.14 g m⁻² year⁻¹ in the manure plots and 5.78 to 10.22 g m⁻² year⁻¹ in the inorganic fertilizer plots (Table 7). Annual production by *Aporrectodea* spp. declined by approximately 50% between 1994–1995 and 1995–1996; and



Fig. 3 Size frequency distribution of *Aporrectodea* spp. in inorganic fertilizer plots during 1994–1996. Width of horizontal bars indicates the relative frequency of occurrence of *Aporrectodea* spp. in each length class. Numbers above histograms indicate the number of earthworms measured at each date

Fig. 4 Size frequency distribution of *Lumbricus* spp. in manure plots during 1994–1996. *Width of horizontal bars* indicates the relative frequency of occurrence of *Lumbricus* spp. in each length class. *Numbers above histograms* indicate the number of earthworms measured at each date

Fig. 5 Size frequency distribution of *Lumbricus* spp. in inorganic fertilizer plots during 1994–1996. *Width of horizontal bars* indicates the relative frequency of occurrence of *Lumbricus* spp. in each length class. *Numbers above histograms* indicate the number of earthworms measured at each date



Sampling date

the *P/B* ratio of *Aporrectodea* spp. ranged from 3.6 to 4.0 (Table 7). The annual production of *Lumbricus* spp. was between 7.44 and 18.11 g m⁻² year⁻¹ and the P/B ratio of *Lumbricus* spp. ranged from 1.9 to 2.0 during the study (Table 7). There was a 40% reduction in *Lumbricus* spp. production in manure plots and a 27% reduction in *Lumbricus* spp. production in inorganic fertilizer plots between 1994–1995 and 1995–1996 (Table 7). The annual production of earthworm communities in corn agroecosystems ranged from 13.22 to 32.25 g m⁻² year⁻¹ (Table 7).

Annual N flux through *Aporrectodea* spp. and *Lumbricus* spp. populations

The N flux through *Aporrectodea* spp. and *Lumbricus* spp. populations in manure plots was between 5.4 and 5.5 g N m⁻² year⁻¹ in 1994–1995 and declined to between 2.8 and 2.9 g N m⁻² year⁻¹ in 1995–1996; depending on the method used to calculate secondary production (Table 8). The N flux through earthworm populations in inorganic fertilizer plots was between 3.0 and 3.4 g N m⁻² year⁻¹ in 1994–1995 and between 1.8 and

Table 6 Secondary production of *Aporrectodea* spp. in manure-amended plots of corn agroecosystems during 1994–1995 calculated by the size frequency (SF) method

| Length class (cm) | Abundance (no. m ⁻²) N | Individual mass (g AFDW) W | No. lost (no. m ⁻²) N | Biomass (g AFDW m ⁻²) ΔN×W | Mass lost (g) \overline{W}_c | Biomass lost (g m ⁻²) $\Delta N_c \times \overline{W}_c$ | Biomass lost×6 $\Delta N \times \overline{W}_c \times h$ |
|---|---|---|---|--|--------------------------------|--|--|
| 0-3.9 | 37 | 0.0071 | 12 | 0.263 | 0.0171 | 0.206 | 1.234 |
| 4-5.9 | 25 | 0.0272 | 3 | 0.679 | 0.0399 | 0.120 | 0.719 |
| 6-7.9 | 22 | 0.0527 | 10 | 1.159 | 0.0698 | 0.698 | 4.190 |
| 8-9.9 | 12 | 0.0870 | 9 | 1.044 | 0.102 | 0.922 | 5.533 |
| 10-11.9 | 3 | 0.1180 | 2.8 | 0.354 | 0.136 | 0.407 | 2.279 |
| 12-13.9 | 0.2 | 0.1534 | 0.2 | 0.031 | 0.153 | 0.031 | 0.184 |
| Annual mea Cohort proc Cohort <i>P/B</i> Annual pro Annual <i>P/B</i> | an biomass= 3.53 duction (CP)= 14 . = 4.0 duction (P)=CP× = 4.0 | g AFDW m ⁻² 14 g AFDW m ⁻² CPI=14.14 g AFDW r | m-2 | | | | |

Table 7 Summary of annual mean biomass, production, and the *P/B* ratio for *Aporrectodea* spp. and *Lumbricus* spp. in corn agroecosystems receiving manure or inorganic fertilizer amendments using the SF method to estimate secondary production

| Year | Species | Biomass | Total production (g AFDW m ⁻² year ⁻¹) | | | |
|------------------------|---|------------------------------|---|--------------------------------|--|--|
| | | (g AFDW m ⁻²) | Cohort production | Annual production | <i>P/B</i> ratio (year ⁻¹) | |
| Manure | | | | | | |
| 1994–1995 1994–1995 | <i>Aporrectodea</i> spp. <i>Lumbricus</i> spp. Total | 3.53 9.78 13.31 | 14.14 36.23 50.37 | 14.14 18.11 32.25 | 4.0 1.9 2.4 | |
| 1995–1996 1995–1996 | <i>Aporrectodea</i> spp. <i>Lumbricus</i> spp. Total | 1.87 5.29 7.16 | 7.11 21.25 28.36 | 7.11 10.63 17.74 | 3.8 2.0 2.5 | |
| Inorganic ferti | lizer | | | | | |
| 1994–1995 1994–1995 | <i>Aporrectodea</i> spp. <i>Lumbricus</i> spp. Total | 2.86 5.28 8.14 | 10.22 20.41 30.63 | 10.22 10.21 20.43 | 3.6 1.9 2.5 | |
| 1995–1996 1995–1996 | Aporrectodea spp. Lumbricus spp. Total | 1.44 3.90 5.34 | 5.78 14.87 20.65 | 5.78 7.44 13.22 | 4.0 1.9 2.5 | |

2.2 g N m⁻² year⁻¹ during 1995–1996 (Table 8). Between 64 and 76% of the annual N flux through earthworm communities in corn agroecosystems was from biomass turnover and the remainder was from N secretion and N excretion.

Discussion

Most studies of earthworm secondary production provide a single estimate of earthworm production, but we have estimates from two methods of calculating production for *Aporrectodea* spp. and *Lumbricus* spp populations in agroecosystems over a 2-year period. It was clear from the size-frequency histograms that cohorts could not be identified easily, and therefore cohort methods to calculate secondary production were not appropriate. We estimated earthworm production with two non-cohort methods, namely the IGR and SF methods. Production by *Aporrect*- *odea* spp. and *Lumbricus* spp. populations in manure and inorganic fertilizer plots during a 2-year period was within the 2.9–44.3 g AFDW m⁻² year⁻¹ that has been reported for earthworm production (Böström 1988; Curry et al. 1995; Parmelee and Crossley 1988; Senapati et al. 1991).

Production estimates for *Aporrectodea* spp. and *Lumbricus* spp. populations with the IGR method were within 3–27% of the values estimated with the SF method. Some of the difference between production estimates from the IGR and SF methods may have been due to the frequency with which earthworm populations were sampled. Populations are sampled at monthly intervals in aquatic production studies (Benke 1984), but earthworm populations were sampled mainly in the spring and autumn. The variability among production estimates might have been reduced had we sampled earthworm populations in the field more frequently. Production estimates from the IGR method may have been improved had we determined earthworm growth more frequently and mea-

Table 8 Summary of the annual N flux through *Aporrectodea* spp. and *Lumbricus* spp. populations in manure and inorganic fertilizer amended plots of corn agroecosystems during 1994–1996 based on IGR and SF methods of estimating earthworm secondary production

| Year | Species | N flux (g N m ⁻² year ⁻¹) | | | |
|------------------------|---|--|-----------------------------|--|--|
| | | IGR method | SF method | | |
| Manure | | | | | |
| 1994–1995 1994–1995 | <i>Aporrectodea</i> spp. <i>Lumbricus</i> spp. Total | 2.60 2.87 5.47 | 2.36 3.01 5.37 | | |
| 1995–1996 1995–1996 | <i>Aporrectodea</i> spp. <i>Lumbricus</i> spp. Total | 0.89 1.86 2.75 | 1.16 1.76 2.92 | | |
| Inorganic fert | tilizer | | | | |
| 1994–1995 1994–1995 | <i>Aporrectodea</i> spp. <i>Lumbricus</i> spp. Total | 1.34 1.60 2.95 | 1.65 1.74 3.39 | | |
| 1995–1996 1995–1996 | <i>Aporrectodea</i> spp. <i>Lumbricus</i> spp. Total | 0.60 1.16 1.76 | 0.89 1.32 2.21 | | |

sured environmental parameters besides soil temperature that might affect earthworm growth.

The similarity in production estimates from the IGR and SF methods supported our use of development times of one year for Aporrectodea spp. and two years for Lumbricus spp. in the SF method. The life histories of few lumbricid earthworm species have been investigated, and most studies determined the development time of earthworms under laboratory conditions (Andersen 1987; Kale and Bano 1994; Haimi 1990). We are aware of only one study that has investigated the development time of L. terrestris under field conditions (Lakhani and Satchell 1970). Butt et al. (1994) found that the time to maturity for L. terrestris hatchlings under optimal laboratory conditions was 30 weeks, while Daniel et al. (1996) found L. terrestris grown at 20°C reached maturity in about 10 weeks. These development times are much shorter than the 2 years we used to estimate production by Lumbricus spp. populations dominated by L. terrestris, and we caution against using laboratory-based development times for this species as it would result in a gross overestimation of Lumbricus spp. production.

The biomass and production of earthworm populations were greater in manure-amended than inorganically fertilized plots during both 1994–1995 and 1995–1996. The manure plots received 8–10 Mg dry matter ha⁻¹ each year in straw-packed cow manure, and we suggested that earthworm populations in the inorganic fertilizer plots were limited by the availability of palatable organic substrates (Whalen et al. 1998). There was a decline in biomass and production of earthworms in both manure and inorganic fertilizer plots between the first and second years of the study. Whalen et al. (1998) attributed the decline in earthworm populations in part to unfavorable climatic conditions during the latter half of 1995; which included a very hot dry summer (mean monthly soil temperatures exceeded 23°C in July and August 1995) followed by the early onset of winter (mean monthly soil temperatures were less than 5°C by November 1995). In addition, the number of *Aporrectodea* spp. had declined by up to 89% and *Lumbricus* spp. numbers had declined by 56–71% in the 5 years since the agroecosystems were converted from alfalfa to continuously cultivated corn.

Production by *Lumbricus* spp. populations tended to be greater than Aporrectodea spp. production in manure and inorganic fertilizer plots. It was interesting that the production of *Aporrectodea* spp. in manure plots during 1994–1995 was only 5–33% lower than Lumbricus spp. production, depending on the method used to estimate production, despite the nearly 62% lower mean annual biomass of Aporrectodea spp. populations. The higher growth rates of *Aporrectodea* spp. than *Lumbricus* spp. contributed to the high production by *Aporrectodea* spp. relative to *Lumbricus* spp. production. It is possible that Lumbricus spp. growth under field conditions was underestimated since the soil in cores used to measure Lumbricus spp. growth had been sieved and repacked. Whalen and Parmelee (1999b) found that L. terrestris created deep vertical burrows in their cores, and suggested that they expended more energy building burrows than for growth and tissue production since field growth rates were much lower than laboratory growth rates. Future work should focus on better methods to quantify earthworm growth rates in the field. We recommend that in ecosystems with several earthworm species, production should be assessed separately for each population to avoid grossly over- or under-estimating total earthworm production.

The P/B ratio of Aporrectodea spp. ranged from 3.2 to 4.6 year⁻¹, depending on the method of calculating production, and was similar to the P/B ratio of 3.3 year⁻¹ reported for earthworm populations dominated by L. rubellus and A. turgida reported by Parmelee and Crossley (1988). Böström (1988) reported a lower P/B ratio of 1.4–1.5 year⁻¹ for A. caliginosa, a related species, which is in the same range as the P/B ratio of Lumbricus spp. populations. The relationship between production (P)and the mean standing stock biomass (B) indicates the biomass turn over rate of populations, while the reciprocal of the P/B ratio indicates the time required to replace biomass in populations (Benke 1984). Biomass replacement time was 78–112 days for Aporrectodea spp. populations and 150–257 days for *Lumbricus* spp. populations. These results suggest that factors causing a decline in earthworm populations would have more profound impacts on *Lumbricus* spp. than *Aporrectodea* spp. populations.

There has been a 87–88% decline in the mean annual biomass of *Aporrectodea* spp. populations and a 82–88% decline in the mean annual biomass of *Lumbricus* spp. populations in the 5 years since the agroecosystems were converted from alfalfa to continuously cultivated corn (P.J. Bohlen, unpublished work). It is interesting that the percent decline was similar for both *Aporrectodea* spp.

and *Lumbricus* spp. populations. Earthworm populations are affected significantly by cultivation (Fraser et al. 1996; Böström and Lofs-Holmin 1995), and the annual cultivation (disk-tillage) of manure and inorganic fertilizer plots to a depth of 15 cm appeared to reduce earthworm populations. Earthworm mortality from mechanical damage during cultivation was likely higher for surface-dwelling earthworms (*A. tuberculata*, *A. trapezoides* and *L. rubellus*) than *L. terrestris*, which could escape damage from tillage by retreating in their burrows. The decline in *Lumbricus* spp. biomass after 5 years of continuously cropped corn may have been related to resource limitation, while the decline in *Aporrectodea* spp. biomass may have been due to cultivation, however this remains to be investigated.

The annual N flux through earthworms was substantial and ranged from 1.76 to 5.47 g N m⁻² year⁻¹ during the study. These estimates are consistent with the N flux of 10–74 kg N ha⁻¹ year⁻¹ through earthworm populations that has been reported (Andersen 1983; Christensen 1987; Böström 1988; Parmelee and Crossley 1988; Curry et al. 1995). The N flux through earthworms was greater in manure than inorganic fertilizer plots due to higher production in the manure plots. The uncertainty in N flux estimates stems from assumptions made regarding earthworm mortality and N excretion rates. Future research efforts should concentrate on better quantification of these parameters to properly assess the ecosystem-level contribution of earthworms to N cycling.

Efficiency quotients for earthworms can be calculated from data on N assimilation and N flux through earthworm populations. Recently, Whalen and Parmelee (1999a) determined the efficiency of nitrogen assimilation of *A. tuberculata* and *L. terrestris* in the laboratory by providing earthworms with ¹⁵N-labeled organic substrates, measuring ¹⁵N tracer incorporated in earthworm tissue after 48 h, and calculating N assimilation efficiencies. The N flux through earthworms is equivalent to N assimilated by earthworms because:

$$A = P + E \tag{8}$$

where A is nitrogen assimilated, P is nitrogen used in the formation of earthworm tissues through growth and reproduction, and E is nitrogen excreted from earthworm tissue in mucus and urine. We estimated that 64-76% of the annual N flux resulted from mortality while the remainder was excreted. The net production efficiency (P/A ratio) is the proportion of N assimilated by earthworms used for growth and production of new tissues, and the P/A ratio of earthworms ranged from 0.64 to 0.74. The assimilation efficiencies (A/C ratio), or N assimilated divided by N consumed, of A. tuberculata and L. terrestris ranged from 0.10 to 0.30, depending on the type of organic substrate that earthworms consumed (Whalen and Parmelee 1999a). Therefore, the gross production efficiency (P/C ratio) of earthworms, based on N flux estimates, might range from 0.06 to 0.22.

Petersen and Luxton (1982) reported that the P/A ratio for energy of earthworms in grassland, forest and moorland ecosystems ranged from 0.09 to 0.36, which is lower than our estimate of the P/A ratio based on N flux. It is not unreasonable for the P/A ratio of earthworms to differ for carbon and nitrogen because the net production efficiency measures the proportion of C or N retained in tissues relative to C or N assimilated. The A/C ratio for carbon of earthworms ranges from 0.02 to 0.15 (Bolton and Phillipson 1976; Dash and Patra 1977; Hutchinson and King 1979; Martin et al. 1992; Rozen 1994), and is lower than the A/C ratio for N of earthworms, which ranges from 0.10 to 0.30 (Binet and Trehen 1992; Bouché et al. 1997; Whalen and Parmelee 1999a). Our results suggest C losses from earthworm tissues through mucus secretion and respiration are greater than N losses from tissues through mucus secretion and urine excretion. We assumed N flux from mortality was equivalent to annual production, which may not be appropriate because earthworm populations have declined each year since agroecosystems were converted from alfalfa to corn production (Whalen et al. 1998). In addition, N losses from mucus secretion and urine excretion could be higher than the modified values we used, and hence the amount of N assimilated that is used for tissue production would be lower than we estimated.

The gross production efficiency (P/C ratio) for energy of earthworms has been estimated to range from 0.01 to 0.04 (Bolton and Phillipson 1976; Böström 1988; Rozen 1994). Persson et al. (1980) estimated the P/C ratios, based on C fluxes, of saprovorous, microbivorous, herbivorous, and carnivorous soil invertebrates to be 0.08, 0.12, 0.16, and 0.24, respectively. We estimate a P/C ratio, based on N fluxes, from 0.06-0.22 for earthworms. If P/C ratios based on C and N fluxes are comparable, our results indicate the gross production efficiency of earthworms to be similar to saprovores, microbivores and herbivores. It is well-documented that earthworms consume organic substrates, including soil organic matter, surface residues, live and dead roots, mycorrhizae, algae, fungi and bacteria (Edwards and Bohlen 1996; Gunn and Cherrett 1993; Lee 1985), and our results support the idea that earthworms derive N required for production of new tissues from many organic resources, including dead plant and animal tissues, microorganisms, and living tissues such as plant roots.

One of the drawbacks of this and other studies that have estimated N flux through earthworm populations is that earthworm mortality is assumed not to be caused by predation. There are numerous predators of earthworms in agroecosystems, including birds, small mammals and predaceous arthropods. Predation is likely a major cause of earthworm mortality in agroecosystems, and the consumption of earthworms by predators results in the incorporation of N from earthworm tissues into predator biomass. Soil-dwelling predators cycle N from earthworms through their biomass through excretion and mortality, and it may be appropriate to consider this N as part of the annual N flux through earthworms. However, when predators consume earthworms and then leave the system (e.g., birds), the N from dead earthworm biomass does not contribute to the annual N flux in the agroecosystem. We are not aware of any studies that have examined this problem, and suggest that research is needed to quantify mortality of earthworms caused by predation and by other factors in agroecosystems.

N released from earthworm tissue through mortality other than by predation and N from mucus secretion and urine excretion may be lost from the system through leaching, denitrification, and volatilization, taken up into plant tissue, or immobilized by microbial biomass. The fate of N released from earthworm tissues through nonpredatory mortality was examined by Whalen et al. (1999) using ¹⁵N-labeled earthworm tissue. About 70% of the N released from dead earthworms had been incorporated into plant shoots after 16 days. About 50% of the N excreted by earthworms in mucus and urine was in mineral N forms (NH₄-N and NO₃-N) while an additional 4-7% was in dissolved organic N forms (Whalen et al. 2000). These studies indicate that a large proportion of the N flux through earthworms is potentially available for plant uptake.

The N content of above-ground plant biomass, including the corn crop and weed biomass, in these agroecosystems was about 185 kg N ha⁻¹ year⁻¹ in 1995 and 160 kg N ha⁻¹ year⁻¹ in 1996 (D.A. McCartney, personal communication). The flux of N through earthworms was equivalent to 16–30% of plant N uptake during 1994–1995 and 11–18% of plant N uptake during 1995–1996. We conclude that the effects of earthworms on N cycling are substantial, particularly in manureamended agroecosystems.

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