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# Impacts of earthworms on soil nutrients and plant growth in soybean and maize agroecosystems

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#### Abstract

The objective of this experiment was to determine the effects of earthworms on soil N pools and plant growth in soybean and maize agroecosystems. The species and number of individuals in earthworm communities were manipulated in plot-scale field enclosures (2.4 m  $\times$  1.2 m) by first reducing earthworm populations within enclosures with carbaryl pesticide, and then adding earthworm treatments to the enclosures. Soybean was grown in the enclosures in the first year and stover maize in the second year.

The success of earthworm manipulations in field enclosures was affected by climate conditions and available food resources. The endogeic earthworm species *Aporrectodea caliginosa* was dominant in all enclosures, while introduced anecic *Lumbricus terrestris* earthworms had poor survival. In the first season, when climate conditions were favourable for earthworm survival and growth, there was a significant (P < 0.05) linear increase in soil mineral-N and microbial biomass N concentrations in the 0–15 cm depth of enclosures with more earthworms. Similarly, soybean grain and grain-N yield was significantly (P < 0.05) greater in enclosures with the largest earthworm populations than the control which had no earthworms added. In the second season, when climate conditions were less favourable, there was no effect of earthworms on soil N pools or maize plants, probably due to poor survival of introduced earthworms.  $\mathbb{O}$  2006 Elsevier B.V. All rights reserved.

Keywords: Earthworms; Population manipulation; Field enclosures; Soil nutrient dynamics

## 1. Introduction

Earthworms are commonly referred to as ecosystem engineers for their ability to modify soils and plant communities (Lavelle et al., 1997; Hale et al., 2005). Through the incorporation of surface litter, casting, burrowing and other activities, earthworms can significantly alter soil physical properties (Edwards and Shipitalo, 1998), soil nutrients (Edwards and Bohlen, 1996), soil biological communities (Doube and Brown, 1998), and above-ground plant communities (Piearce et al., 1994; Wurst et al., 2005).

The functional relationships between earthworms, soils and plants have been extensively studied in microcosm and laboratory experiments. However, extrapolating these results to the ecosystem-level is difficult. Earthworm activities may be overstated in small-scale experiments due to the control of environmental variables like temperature, soil moisture and food availability or because an unrealistic number of earthworms are added to small containers or mesocosms. The challenge is to quantify the influence of realistic earthworm communities at the field-level (Bohlen et al., 2004), which is often done by manipulating earthworm populations and communities in large-scale field enclosures (see Bohlen et al., 1995; Baker et al., 1996; Subler et al., 1997). However, there is considerable variation in the success of earthworm manipulations in field enclosures, depending on the methods used, climate and soil conditions (Bohlen et al., 1995; Baker et al., 1996; Zaller and Arnone, 1999; Emmerling and Pausch, 2001).

Therefore, the objectives of this experiment were (1) to determine the effects of an earthworm community, dominated by *A. caliginosa* and *L. terrestris*, on soil nutrient dynamics and plant growth in soybean and maize agroecosystems, and

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communities by reducing the population with pesticide and adding earthworms belonging to different functional groups.

## 2. Materials and methods

The study was conducted from May to September in 2004 and 2005 on the Research Farm of Macdonald Campus of McGill University, Quebec, Canada ( $45^{\circ}25'$ N,  $73^{\circ}56'$ W). The field was used for soybean and maize production in the 2 years prior to this experiment and before that was a turfgrass sports field. The soil was a mixed, frigid Typic Endoquent, classified as a Chicot series sandy loam. It had a pH (H<sub>2</sub>O) of 5.9, an organic C content of 24.5 g C kg<sup>-1</sup>, and contained 580 g kg<sup>-1</sup> sand, 300 g kg<sup>-1</sup> silt, and 120 g kg<sup>-1</sup> clay. A field survey in May, 2003 found an earthworm community with an average of 50 individuals m<sup>-2</sup> of *A. caliginosa* and 15 individuals m<sup>-2</sup> of *L. terrestris*, and age class ratios of juveniles to adults of 4:1 and 3:1, respectively.

Field enclosures were installed in April, 2004. These rectangular sheet metal enclosures measured  $2.4 \text{ m} \times 1.2 \text{ m}$  (2.9 m<sup>2</sup>) and were buried to a depth of 0.30–0.40 m. 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. The enclosures remained in place for the 2004 and 2005 seasons.

At the beginning of each season, carbaryl pesticide (Sevin<sup>®</sup>) was applied to reduce earthworm populations in the enclosures. Beginning on April 28th, 2004, carbaryl was applied five times during a 25-day period, giving a total load of about 0.02 kg a.i.  $m^{-2}$ . The next year, we began on April 16th, 2005, and applied carbaryl four times during a 35-day period for a total load of about 0.04 kg a.i.  $m^{-2}$ . In both years, the last application of carbaryl was made 10 days before adding earthworms to the enclosures.

On May 28th, 2004, a single row of 100 soybeans (Glycine max (L.) cv. Merril) was sown by hand lengthwise, in the centre of each enclosure (equivalent to a planting density of 350,000 plants ha<sup>-1</sup>). Germination and seedling establishment was even across all treatments, except in one enclosure. Here we planted 30 additional seeds and thinned to a similar density as the other enclosures within 3 weeks of the original sowing date. On June 1st, 2005, a single row of 15 silage maize (Zea mays (L.) cv. Mycogene 2K350) seeds were sown by hand lengthwise, in the centre of each enclosure (equivalent to a planting density of 52,000 plants ha<sup>-1</sup>). Germination and seedling establishment was uneven and additional seeds were planted 7 days later. After 2 weeks, we thinned to 12 plants per enclosure. No fertiliser or pesticide was added to either crop. Weeds were removed by hand as required throughout the season.

# 2.1. Experimental design

The experiment was a randomised complete block design with seven earthworm population treatments and four

blocks. The seven earthworm population treatments were three combinations of earthworms as A. caliginosa only (A), L. terrestris only (L), and a combined A. caliginosa and L. terrestris treatment (AL), at either a background population level  $(1 \times)$  or double the background population level  $(2 \times)$ , and a control treatment with a reduced earthworm population. In the 1-4 weeks before the experiment began, earthworms were collected from around the field site and nearby arable fields by hand-sorting and formalin extraction (Raw, 1959). The earthworms were sorted by species and age-class and kept in laboratory cultures (381 plastic bins) containing soil from the field site, regularly watered and fed with composted cattle manure. The mean fresh weight biomass of earthworms added to enclosures was similar in both years. In 2004 the fresh weight biomass of adult and juvenile A. caliginosa was  $0.48 \pm 0.19$  g and  $0.31 \pm 0.11$  g, respectively, and  $4.79 \pm 1.07$  g and  $1.53 \pm 0.87$  g for adult and juvenile L. terrestris, respectively. In 2005 the fresh weight biomass of adult and juvenile A. caliginosa was  $0.59 \pm 0.27$  g and  $0.24 \pm 0.13$  g, respectively, and  $4.72 \pm 0.86$  g and  $1.87 \pm 0.99$  g for adult and juvenile L. terrestris, respectively. The ratio of juvenile to adult earthworms added to enclosures in both years was 1.5 for A. caliginosa and 3.9 for L. terrestris. In both years, we attempted to add earthworms to the enclosures on a cloudy overcast day; June 1st in 2004, and June 6th in 2005. Earthworms were transported to the field in 11 pots, each containing 10-30 earthworms in about 100 g of moist field soil. The earthworms in each pot were spread evenly in two trenches (5–10 cm deep), dug lengthwise in the enclosures. The earthworms were then lightly covered with soil and about 71 of water was poured evenly along the trenches. Straw was lightly placed above the trenches to provide additional protection from direct sunlight and predators. The straw was removed 3 days later. The number and biomass of earthworms added to each treatment in June and collected in

Table 1

Earthworm populations and biomass added in June 2004 and collected in October 2004 from enclosures under soybean production<sup> $\dagger$ </sup>

Earthworm treatment	Population (individuals $m^{-2} \pm S.E.$ )		Biomass (g fresh weight $m^{-2} \pm S.E.$ )	
	June	October	June	October
Control	0	$77\pm12$ b	0	$25\pm7.5$ b
A1x	50	$190\pm56~\mathrm{ab}$	21	$56\pm15~ab$
A2x	100	$330\pm87$ a	42	$86\pm22$ ab
L1x	15	$170\pm24$ ab	34	$55\pm9.2$ ab
L2x	30	$220\pm46~\mathrm{ab}$	67	$77 \pm 12$ ab
AL1x	65	$180\pm 62~ab$	55	$86\pm25~ab$
AL2x	130	$380\pm47$ a	109	$92\pm8.7$ a
Background <sup>††</sup>		$123\pm30$		$59\pm4.5$
ANOVA treatr	nent effects			
Treatment		P = 0.01		P = 0.03

<sup>†</sup> Values in each column for each treatment followed by similar letters are not significantly different by Tukey's HSD test (P = 0.05).

<sup>††</sup> Background samples were the average of two pits and therefore were not included in the statistical analysis.

Table 2 Earthworm populations and biomass added in June 2005 and collected in October 2005 from enclosures under silage maize production

Earthworm treatment	Population (individuals $m^{-2} \pm S.E.$ )		Biomass (g fresh weight $m^{-2} \pm S.E.$ )	
	June	October	June	October
Control	0	$93\pm18$	0	$23\pm5.4$
A1x	50	$132\pm23$	24	$34\pm7.1$
A2x	100	$135\pm33$	47	$25\pm8.4$
L1x	15	$147\pm67$	37	$44\pm19$
L2x	30	$117 \pm 45$	75	$33 \pm 10$
AL1x	65	$153\pm24$	61	$43 \pm 11$
AL2x	130	$95\pm10$	123	$44 \pm 20$
Background <sup>†</sup>		$233\pm15$		$118\pm3$
ANOVA treatr	nent effects			
Earthworm		n.s.		n.s.

<sup>†</sup> Background samples were the average of two pits and therefore were not included in the statistical analysis.

October in 2004 and 2005 are presented in Tables 1 and 2. We considered the results from each season independently since carbaryl was applied to reduce the earthworm populations in both years, before treatments were applied. To avoid the confounding effect of previous earthworm manipulation during the 2005 season, the earthworm treatments within each block were re-randomised in April 2005.

#### 2.2. Plant, earthworm and soil analysis

In 2004, five soybean plants from each enclosure were carefully uprooted 6, 10 and 14 weeks after sowing. In week 6 and 10 only, plant roots were washed and the root dry weight and number of Rhizobium nodules recorded. On September 29th, about 18 weeks after sowing, the shoots of 20 soybean plants were cut at the soil surface from each enclosure. Shoot dry weights were determined in all weeks, and grain dry weights recorded in week 18 only. On August 2nd, 2005, about 9 weeks after sowing, six maize leaves per enclosure were taken for nutrient analysis. On September 26th, all 12 maize plants were harvested from each enclosure by cutting shoots at the soil surface and the shoot, cob and grain dry weights were determined. In both years, subsamples were taken from all shoot, leaf and grain samples and ground with a Wiley mill (<1 mm mesh). Plant shoot, leaf and grain sub-samples were digested with H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> (Parkinson and Allen, 1975) and digests were analysed colorimetrically for N and P using a Lachat Quick Chem autoanalyzer (Lachat Instruments, Milwaukee, WI, USA), and for K using atomic absorption spectrometry. N-yield of soybean grain and maize plants were determined on a plant specific basis by multiplying the grain or tissue N concentration by the grain or total weight for soybean and maize plants, respectively.

In both years, earthworm populations were sampled from a soil pit (50 cm  $\times$  30 cm to a depth of 20 cm) dug in the middle of each enclosure 1–5 days after plant harvest. The

removed soil was hand sorted for surface-dwelling earthworms, and formalin extraction (Raw, 1959) was used to collect earthworms from lower depths beneath the pit. Earthworm numbers, age classes, and fresh biomasses of earthworms were later recorded in the lab. Sexually mature individuals were identified to the species level using the key provided by Reynolds (1977). In May 2005, earthworm populations were sampled using the same method described above from a soil pit (15 cm × 15 cm to a depth of 20 cm) dug in the middle of each enclosure 1 week prior to adding earthworms.

In both years, soils from each enclosure were sampled 2– 3 days after plant harvest. Four soil cores were taken diagonally across each enclosure from two depths (0-15 cm and 15–30 cm) with a soil auger (2 cm internal diameter) and composited into one sample per depth per enclosure. Soil samples were kept at 4 °C until laboratory analysis. Mineral nitrogen  $(NO_3-N + NH_4-N)$  was determined by extracting 5 g field-moist soil with 50 ml of 2 M KCl (Maynard and Kalra, 1993). After shaking for 1 h and filtering, the extract was analysed by colorimetry for NO<sub>3</sub>-N and NH<sub>4</sub>-N on a Lachat Quick Chem auto-analyser (Lachat Instruments, Milwaukee, WI, USA). Microbial biomass nitrogen (MBN) in soil samples was analysed using the chloroform fumigation-direct extraction method followed by persulfate digestion and calculated as: [(total extractable N after fumigation – total extractable N before fumigation)/ 0.54] (Brookes et al., 1985; Joergensen and Mueller, 1996). Dissolved organic nitrogen was calculated as the difference between the NO<sub>3</sub>-N and NH<sub>4</sub>-N concentrations in a persulfate digested soil extract and the original undigested soil extract (Cabrera and Beare, 1993). Available P and K were determined by extracting 2.5 g air-dry soil with 25 ml Mehlich-III solution (Tran and Simard, 1993). The P concentration in extracts was analysed colorimetrically on a Lachat Quick Chem auto-analyser (Lachat Instruments, Milwaukee, WI, USA) and K concentration was measured using atomic absorption spectrophotometry.

# 2.3. Statistical analysis

The effects of earthworm treatment on soil properties, soybean nodulation, plant nutrients and yields were evaluated by one-way analysis of variance using the PROC GLM function of SAS software (SAS Institute Inc, 2001). The differences between least square means of significant treatment effects were evaluated using the Tukey–Kramer HSD test (P = 0.05). Regression lines were fitted using the PROC REG function of SAS software (SAS Institute Inc, 2001).

## 3. Results

Daily temperature fluctuations and weekly rainfall patterns in 2004 were similar to the 30-year mean for the

region (Environment Canada, 2005). More extreme temperature and precipitation events were observed in 2005. In 2005, above normal temperatures began in early June and continued for the next 12 weeks. There were 8 days in June and 9 days in July, 2005 with a maximum temperature of over 30 °C. In contrast, the 30-year mean indicated 1.6 days in June and 4 days in July with a maximum temperature above 30 °C. In 2004, temperatures were cooler than normal, with no days in June and only 1 day in July with a maximum temperature of over 30 °C. The total precipitation during the experiment was similar in 2004 (305 mm) and 2005 (404 mm) to the 30-year mean (395 mm). However, the frequency of rainfall events was low in 2005 but the average rainfall per event was greater. Weekly rainfall tended to be lower than long term averages in June and July of 2005, except for three above-average rainfall events totalling 178 mm. In the week following earthworm introductions in 2004, temperatures ranged from 9 °C to 25 °C with a mean temperature of 15 °C, and total precipitation was 36 mm in the week following earthworm introduction. In 2005, temperatures ranged from 14 °C to 33 °C with a mean temperature of 23 °C, and total precipitation was only 6 mm in the week after earthworms were placed in enclosures.

In 2004 and 2005 the manipulation of species and abundance of earthworms in each treatment was not successful. Although significant differences in earthworm population (P < 0.01) and biomass (P < 0.03) recovered in October were found between treatments in 2004 (Table 1), there were no significant differences in the number of earthworms of each species recovered in October between treatments in 2004 or 2005 (data not shown).

In October 2004, the earthworm community in all enclosures was dominated by *A. caliginosa* (59–100%) with a few *L. terrestris* (0–33%) and *Aporrectodea longa* (0–23%), and in October 2005 it was dominated by *A. caliginosa* (56–100%) with a few *L. terrestris* (0–44%) and *A. longa* (0–8%).

In May 2005, after three applications of pesticide and before the addition of earthworms, the populations within enclosures were reduced to 32 individuals  $m^{-2}$  with a fresh weight biomass of 3 g  $m^{-2}$ , on average. There was no difference between enclosures applied the previous year suggesting that the re-randomising of treatments was not biased by the earthworm treatments.

Even though the manipulation of the abundance of different earthworm species was not successful, the manipulations did achieve a wide range of population and biomass across all enclosures. In 2004, the earthworm populations in enclosures ranged from 53 to 553 individuals  $m^{-2}$  and biomass ranged from 11 to 159 g fw  $m^{-2}$ , and in 2005 the populations ranged from 33 to 347 individuals  $m^{-2}$  and biomass ranged from 9 to 104 g fw  $m^{-2}$ .

In 2004, a significant increasing linear relationship was found between earthworm numbers and NO<sub>3</sub>-N (P = 0.01), NH<sub>4</sub>-N (P = 0.03), and MBN (P < 0.001) concentrations, and between earthworm fresh-weight biomass and MBN



Fig. 1. Relationship between soil mineral-N concentration (mg N kg<sup>-1</sup>) in the 0–15 cm depth and earthworm population under soybean production in 2004 (n = 28).

(P = 0.008) in the 0–15 cm soil depth. A decreasing linear relationship was found between earthworm numbers and the DON (P = 0.017) concentration in the 0–15 cm soil depth. No significant relationships were found between earthworm numbers, biomass and soil nutrients in the 15–30 cm depth. The relationships between earthworm numbers and the mineral-N (NO<sub>3</sub>-N + NH<sub>4</sub>-N), and MBN concentrations in the 0–15 cm depth are presented in Figs. 1 and 2. In 2005, there were no significant relationships between earthworm numbers or biomass and soil nutrient concentrations at both depths.

In 2004, soybean grain yield ranged from 15.7 to 28.8 g plant<sup>-1</sup>, and total harvested yield ranged from 44.2 to 74.8 g plant<sup>-1</sup>. Significant logistic relationships were found between earthworm numbers and total grain-N per plant (P = 0.002) (Fig. 3) and grain yield (P = 0.036), and earthworm fresh-weight biomass and total grain-N per plant (P = 0.004), grain yield (P = 0.016), and total yield (P = 0.03). No relationships were found between earthworm numbers or biomass and the number of nodules per plant at 6 and 10 weeks, and nutrient concentrations in grain at harvest.

In 2005, maize grain yield ranged from 62.5 to  $184 \text{ g plant}^{-1}$ , and total silage yield ranged from 184 to



Fig. 2. Relationship between MBN concentration (mg N kg<sup>-1</sup>) in the 0– 15 cm depth and earthworm population under soybean production in 2004 (n = 28).



Fig. 3. Relationship between total grain-N per soybean plant (g N plant<sup>-1</sup>) and earthworm population in 2004 (n = 28).

384 g plant<sup>-1</sup>. However, no relationships were found between earthworm numbers or biomass and nutrient concentrations in stover and grain, total N yield per plant at harvest, and grain, stover and total silage yield.

# 4. Discussion

The manipulation of earthworm functional groups was not successful in both years. A. caliginosa earthworms were most numerous in all treatments indicating that earthworms or cocoons of this species survived better after carbaryl application than those of other species. The average number of L. terrestris in all treatments did not differ significantly in both years (24 versus 29 individuals  $m^{-2}$  in 2004 and 2005, respectively) and were similar to the average number found in background samples (17 and 30 individuals  $m^{-2}$  in 2004 and 2005, respectively). Moreover, in both years, treatments with introduced L. terrestris had the same number as those with no introduced L. terrestris. This may indicate high mortality of introduced L. terrestris and persistence of the initial L. terrestris population and cocoons, even after several applications of carbaryl pesticide. The number of A. longa earthworms in enclosures decreased from an average of 19 to 2 individuals  $m^{-2}$  between the first and second years, indicating that it may take longer than 2 years to eliminate non-introduced species from enclosures with carbaryl pesticide.

The introduction of earthworms, regardless of functional group, was more successful in 2004 than in 2005, even though earthworms were added on almost the same day each year. A combination of higher temperatures, lower precipitation and lower food availability may have led to greater mortality when earthworms were introduced in a 2005 than 2004.

The effectiveness of carbaryl applications at reducing the naturally occurring earthworm populations was not very consistent. Carbaryl applications did not completely eliminate earthworms from the enclosures, and the earthworms that persisted were probably as active and capable of growing and reproducing as introduced earthworms. However, the control treatments were similar in both years and had the fewest number of earthworms, from 77 to 93 individuals  $m^{-2}$ , and lowest biomasses, ranging from 23 to 25 g fw  $m^{-2}$  of all enclosures. Furthermore, the earthworm populations within control enclosures were similar to populations at locations adjacent to the enclosures (background population) in 2004 but not 2005 (Tables 1 and 2). In sandy loam soils, such as at our field site, carbaryl has a half-life of about 4–7 days (Venkateswarlu et al., 1980). Nonetheless, carbaryl can reduce earthworm numbers and biomass by up to 90% and these low numbers persist for up to 3 weeks after application (Potter et al., 1990; Vangestel, 1992).

In other field studies, earthworm community manipulations have had varied success. In pastures of south-eastern Australia, the introduction of earthworms into 30 cm diameter cores has generally shown moderate survival of introduced species (50-80%), a high number of nonintroduced species (25–200 individuals  $m^{-2}$ ) and varied population growth ranging from 0.5 to 3 times the introduced population (Baker et al., 1996, 1999a,b, 2002; Chan et al., 2004). In Ohio, USA, earthworms introduced over a 3-year period into large  $4.5 \times 4.5$  m field enclosures had high mortality since populations grew by only 12-22% even though the total population added over 3 years was three times greater than the final population (Bohlen et al., 1995). In another earthworm manipulation study in Ohio, earthworm populations did not differ between increased and unmodified population treatments 5 months after earthworms were introduced into  $6.1 \text{ m} \times 6.1 \text{ m}$  enclosures (Subler et al., 1997). Similar low survival rates were reported by Boyer et al. (1999) on Reunion Island, where only about 10% of earthworms added to  $6.0 \text{ m} \times 1.5 \text{ m}$ enclosures survived 7 months after introduction. Emmerling and Pausch (2001) had better success in Germany where earthworm populations introduced into  $1.4 \text{ m} \times 0.9 \text{ m}$ enclosures increased between two to five fold over 2 years. Similarly, 2 years after adding earthworms to  $1 \text{ m} \times 1 \text{ m}$ enclosures in a Swiss grassland, populations were about 50% greater than ambient populations, and ambient populations were about twice the size of reduced populations (Zaller and Arnone, 1999). In all of these studies, the success of earthworm manipulations into field enclosures was not consistent for any particular species or functional group.

The effect of earthworms on soils differed in each year. In 2004, our results indicate that the size of the earthworm population was related positively to the total mineral-N (NO<sub>3</sub>-N + NH<sub>4</sub>-N) and MBN concentrations after harvest (September 2004). The relationship described in Fig. 1 suggests that an individual earthworm can increase the soil mineral-N pool by 0.02 kg N m<sup>-2</sup>. Expressed in more tangible terms, a field with a high earthworm population (300 individuals m<sup>-2</sup>) could have 14 kg N ha<sup>-1</sup> more in the 0–15 cm soil depth than a field with a low population (30

individuals  $m^{-2}$ ). We assume that most of this mineral-N was generated by the activities of the endogeic *A. caliginosa* since they were the dominant species at our site. The greater amounts of mineral-N in soils suggest that high earthworm populations in the autumn may increase the risk of N leaching from soils after harvest. However, the increase in MBN also suggests that some of the increase in available-N was being captured in the microbial biomass.

In 2005, a wide range of earthworm populations and biomass was found across all enclosures as a result of earthworm manipulations, yet there were no relationships between earthworm population, biomass and soil nutrients. This important result suggests that the effect of earthworms on soil N dynamics cannot be predicted by earthworm population or biomass alone.

Previous studies investigating the effect of earthworm additions on soil nitrogen dynamics gave mixed results. In a maize-based enclosure study in Ohio, Blair et al. (1997) found that the addition of earthworms increased the soil NO<sub>3</sub>-N concentration over a 2-year period in inorganically fertilized plots but not in manure or legume fertilized plots. Furthermore, they found that earthworm addition had increased soil NO<sub>3</sub>-N concentration at lower depths (15-45 cm) in two consecutive growing seasons and in the 0-15 cm depth in only one of the two growing seasons. In contrast, results from a mesocosm experiment by Bohlen and Edwards (1995) show that earthworms increased the amount of NO<sub>3</sub>-N at the 0-5 cm depth but had no effect at the 5–15 cm depth. In another enclosure study in Ohio, the addition of earthworms did not increase mineral-N concentrations in maize-soybean or maize-soybean-wheat systems in the 0-45 cm depths but did increase pools of organic N (MBN and DON) (Subler et al., 1997). This is consistent with our results of greater MBN concentrations in the 0-15 cm soil depth with increasing earthworm population; however, DON concentrations decreased as earthworm population size increased.

Soybean and silage maize responded differently to earthworm populationns. When weather conditions were more favourable for earthworm activity, as under soybean production in 2004, there were more noticeable effects of earthworms on plant growth and nutrient uptake. Regression analysis shows that soybean grain yield could be 25% greater and the total N removed in soybean grain (g N per plant) could be 40% greater in fields with high earthworm populations (>400 individuals m<sup>-2</sup>) than in fields with low earthworm populations (<50 individuals m<sup>-2</sup>) (Fig. 3).

A lack of response by maize to earthworm activity has been found in other field and greenhouse studies (Mackay and Kladivko, 1985; Stinner et al., 1997; Boyer et al., 1999). The differences between the effects of earthworms on soybean and maize growth may be partially related to the N requirements and rooting pattern of each plant. The recommended N fertiliser requirements of silage maize  $(120-170 \text{ kg N ha}^{-1})$  are much greater than soybeans (0 $30 \text{ kg N ha}^{-1}$ ) due to the N fixation ability of soybeans (CRAAO, 2003). Therefore, any contribution of nitrogen from earthworms will supply a much greater proportion of the recommended N for soybeans than for maize. Furthermore, soybean plants have three times greater proportion of thin roots (<0.12 cm) than maize plants in the 0–10 cm soil depth and the root dry matter density per soil unit volume is greater for maize than soybean plants at lower depths (Venzke et al., 2004). The greater proportion of thin roots of soybean plants in the 0-10 cm depth provides greater contact with the soil matrix, enabling greater nutrient and water uptake. Since the effects of earthworms on soil nutrients in our experiment occurred in the 0-15 cm depth, the shallow rooted soybeans would probably benefit more from earthworm activities that increase soil-N pools than the deep rooted maize.

# 5. Conclusions

Our research suggests that earthworms contribute positively to plant growth in some agroecosystems only when populations are high (>300 ind.  $m^{-2}$ ) and when favourable weather conditions exist. Although we did not detect significant differences between the endogeic and anecic earthworm treatments, a higher earthworm population, principally consisting of the endogeic species *A. caliginosa*, was associated with greater mineral-N and MBN concentrations in surface (0–15 cm) soils, and higher grain N yield in soybeans. A combination of poor survival of introduced earthworms and low activity of the surviving earthworms may explain the lack of effects of earthworms on maize plants.

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