Ecosystems (2004) 7: 672–685
DOI: 10.1007/s10021-004-0150-7

ECOSYSTEMS

Earthworms Increase Nitrogen Leaching to Greater Soil Depths in Row Crop Agroecosystems

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ABSTRACT

Many biological functions of soil organisms are replaced in intensive agricultural systems, but earthworms and other soil invertebrates may continue to have significant effects on nutrient cycling in these disturbed systems. We investigated the influence of earthworms on leaching of water and nitrogen in corn (*Zea mays* L.) agroecosystems in a long-term (6-year) field experiment in Wooster, Ohio, USA. We employed a split-plot experimental design in which main plots received one of three nutrient treatments (cow manure, legume–grass mixture, inorganic fertilizer) and contained three 4.5 × 4.5-m field enclosures in which earthworm populations were increased, decreased, or unmodified. We installed zero-tension lysimeters beneath enclosures with increased or decreased populations and collected leachates regularly in 1996, analyzing them for water volume and concentrations of NH_4^+, NO_3^-, and dissolved organic nitrogen (DON). Earthworms did not influence concentrations of inorganic N or DON but greatly increased leachate volume. The total flux of N in soil leachates was 2.5-fold greater in plots with increased earthworm populations than in those with decreased populations. Earthworm population density was positively correlated with total N leaching flux ($r^2 = 0.49$). Leaching losses of N to a depth of 45 cm were greater in the inorganically fertilized than in the organically fertilized plots, possibly due to greater inorganic N concentrations and lower immobilization potential in inorganically fertilized systems. Our results indicate that earthworms can increase the leaching of water and nitrogen to greater soil depths, potentially increasing N leaching from the system.

Key words: earthworms; continuous corn agroecosystems; nitrogen leaching; groundwater nitrate contamination; water infiltration; nitrogen fertilizer; manure; cover crops; soil microbial biomass; long-term experiments.

INTRODUCTION

The loss of nitrogen (N) from agricultural systems is a major global environmental concern (Vitousek and others 1997; Jenkinson 2001). Application of nitrogen fertilizer to croplands in excess of crop demand or at a time when uptake is not maximal is often associated with nitrate leaching from soil which can lead to increased nitrate concentrations in groundwater and surface water and to potentially adverse consequences for human health and the integrity of downstream ecosystems (Hallberg 1989; Weil and others 1990; Hallegraeff 1993; Howarth and others 1996). Concerns over the use of nitrogen in agriculture require a better understanding of the biological, chemical, and physical factors controlling N loss and the relative impact of contrasting crop production practices on NO_3 leaching from agroecosystems.

Received 25 February 2002; accepted 3 July 2003; published online 23 June 2004.

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Earthworms are a major component of the soil biological community in many agroecosystems in which they can have an important influence on soil processes, but it is not well known whether, and to what degree, earthworms can influence nutrient leaching or loss from these systems (Edwards and Bohlen 1996; Parmelee and others 1998). The transport of water and nutrients through soil macropores and, in particular, through earthworm burrows has received increasing attention as an important mechanism of solute transport from the soil surface to the subsoil and groundwater (Zachmann and others 1987; Shipitalo and others 1990; Edwards and others1992B, 1992C; Trojan and Linden 1992; Stehouwer and others 1994). Earthworm burrows can create preferential flow pathways in the soil profile, thereby altering water balance and water movement (Tomlin and others 1994). Infiltration through these channels is important in influencing the transport of water, agricultural chemicals, and nutrients through the soil (Edwards and others 1989, Edwards and others 1992a; Shipitalo and Edwards 1993). Despite considerable mechanistic research on the influence of earthworms on leaching at the scale of individual burrows or small soil blocks, it is not known whether they can affect nutrient leaching at the ecosystem scale.

Earthworm populations vary widely in different agricultural systems so it is essential to understand whether differences in earthworm populations can influence overall leaching of nutrients from these systems. Differences in tillage, nutrient inputs, and crop rotation can influence the size and species composition of earthworm populations (Edwards and Bohlen 1996). A variety of agricultural land-use practices, such as reduced or no tillage, the addition of organic amendments, and certain crop rotations, increases earthworm populations; whereas other practices, such as intensive tillage, toxic pesticides, and residue removal, decrease earthworm populations (Lofs–Holmin 1983; Berry and Karlan 1993; Hendrix 1998; Edwards and Bohlen 1996). The increasingly widespread adoption of no-tillage agriculture (Allmaras and others 1994) may be contributing to increases in earthworm populations over wide areas and it is important to understand how these changes may contribute to leaching of water and nutrients in these areas.

Long-term, manipulative field experiments provide an opportunity to assess the overall effects of earthworms on N dynamics (Blair and others 1994; Edwards and others 1994). There is a considerable amount of data on the shorter-term effects of earthworms on N dynamics at the microsite level and on soils and soil–plant relationships in laboratory and greenhouse incubations. Less is known about the extent to which similar effects occur in the field, or whether they are of a large enough magnitude to influence overall nutrient fluxes in the field.

We conducted a large-scale, long-term (1991–1998) field experiment in which we manipulated earthworm populations in continuous corn fields receiving different forms of nutrient inputs to investigate the role of earthworms in nutrient cycling in agroecosystems (Blair and others 1995; Bohlen and others 1995b). Early results from this experiment showed that earthworms tended to increase the availability of soil N, and that they may have increased concentrations of soil nitrate at the 30–45-cm depth in the same field plots used in the current study (Bohlen and Edwards 1995; Blair and others 1997). Although these previous results pointed to the potential for earthworms to increase leaching loss of N from the system, they did not provide quantitative evidence that earthworm populations influenced N leaching. Our objective in the current investigation was to quantify the leaching flux of water and nitrogen in plots with artificially increased or decreased earthworm populations in corn agroecosystems based on different long-term (6-year) inputs of inorganic or organic nutrient inputs. This study provides an integrated view of how soil fauna and nutrient input management interact to influence nutrient leaching from row-crop agroecosystems.

METHODS

Study Site

The experiment was set up at the Ohio Agricultural Research and Development Center of Ohio State University in Wooster, Ohio, USA (41°N, 82°W). Mean monthly temperatures at the site range from −4.8°C in January to 21.2°C in July, and the mean annual precipitation is 905 mm/year. The experimental site is a relatively flat area on a fine, mixed, mesic Fragiudalf soil of the Canfield series (a Luvisol in the FAO soil classification). Canfield soils are deep, gently sloping, moderately to well-drained silt loam soils on uplands, with a relatively impermeable fragipan at a depth of 40–75 cm, and represent a major agricultural soil type in the region. The mean percentage organic matter at the site, determined by a modified rapid dichromate oxidation technique (Walkley and Black 1934), was 3.7 ± 0.9% at the outset of the experiment and the soil texture was 13.5% sand, 73.7% silt, and 12.8% clay. Soil pH determined in water (1:1 ratio of soil to water) was 6.3 ± 0.4 and cation exchange capacity was 0.1 ± 0.02 mol_c/kg soil. The site was planted with corn annually between 1984 and 1987, used for alfalfa (*Medicago sativa* L.) production between 1987 and 1991, and planted again annually with corn during this experiment from 1991 to 1998.

Experimental Design

Zero-tension lysimeters for measuring leaching of water and nutrients were installed in the summer of 1995 at a depth of 45 cm in field plots of a larger experiment, begun in 1991, established to investigate the role of earthworms in carbon and nitrogen cycling processes in corn agroecosystems based on organic or inorganic nutrient inputs (Blair and others 1995; Bohlen and others 1997). The experiment was set up in a randomized complete block split-plot design, with three different earthworm population manipulations nested within each of three different fertilizer treatments. The main plots, which were 20 × 30 m, received one of three nutrient applications, applied just prior to spring tillage, at a rate of 150 kg N ha^{-1}. (1) inorganic N (granular NH_4NO_3), (2) straw-packed cow manure, or (3) a legume–rye (*Vicia villosa* Roth–*Secale cereale* L.) mixture cover crop harvested from another area and added as dry plant material to the experimental plots. The legume–grass mixture was used in lieu of growing a cover crop within the plots to allow for greater control over the amount of N added to the plots and because it was difficult to establish the cover crop following corn harvest. We established four block replicates with three fertilizer treatments in each, giving a total of 12 main plots. In 1996 when lysimeter measurements were made, fertilizers were added in early June after the fields became workable and the groundwater levels lowered following very wet conditions in the spring of that year.

Each of the 12 main nutrient–treatment plots contained three 4.5 × 4.5-m field enclosures constructed from PVC sheets (45 cm deep, 15 cm above surface) in which earthworm populations were (i) increased, (ii) decreased, or (iii) left unmodified (control). Twenty cm wide strips of metal screen attached to the upper edge of the enclosures prevented movement of earthworms into or out of the plots. Prior to the start of lysimeter measurements, several small holes were cut in the enclosure walls at the downslope side of the enclosures to allow surface runoff to drain naturally and prevent any buildup of artificial head gradients as a result of the enclosure walls. Only plots with increased and decreased populations were used for the nutrient leaching study to maximize the chance of observing a difference between earthworm population treatments and because resources were not available to install and monitor lysimeters beneath all 36 enclosures.

Earthworm populations were manipulated once or twice (spring and/or autumn) each year from 1991 to 1997 by using an electroshocking technique (which brings most worms to the surface and may also kill some *in situ*) to decrease earthworm populations, and by adding earthworms collected from adjacent areas to increase populations (Bohlen and others 1995b). Preliminary tests and regular sampling and extraction for other soil-inhabiting invertebrates (nematodes, enchytraeids, microarthropods) showed that those groups were not affected significantly by electroshocking (Blair and others 1995).

Earthworm Population Assessment

We sampled earthworm populations once per year in the enclosures using either hand sorting or a combination of hand sorting of the upper 25 cm of soil combined with formalin expulsion to extract deeper-burrowing species (Bohlen and others 1995b). The total plot area affected by earthworm sampling in the plots over a five-year period prior to the lysimeter measurements was less than 1 m^2, which we consider to be negligible relative to other measurements made in the plots. Earthworm populations in 1996, during the period of lysimeter measurements, were assessed by hand sorting, but not formalin expulsion, in each enclosure in the spring (Apr. 22) and fall (Sept. 26). Hand sorting involved excavating 0.05-m^2 blocks of soil (32 cm × 16 cm × 25 cm deep) and sifting through the excavated soil by hand for earthworms. Collected earthworms were immediately placed in 4.0% formalin and returned to the lab where they were identified to species. The earthworms were then oven-dried at 60°C, ground with a mortar and pestle, and ashed in a muffle furnace at 450°C for 4 h. Earthworm biomass was expressed as ash-free dry mass (AFDM) to correct for any soil contained in the earthworm's digestive tract. The hand-sorting method was not adequate to assess populations of the deep-burrowing species, *L. terrestris,* which in 1993 accounted for the majority of earthworm biomass at the site and were disproportionately more abundant in plots with increased earthworm populations (Bohlen and others 1995b). Adult individuals of this species can be sampled only with behavioral techniques, such as formalin expulsion (Edwards and Bohlen 1996), but we did not use this method to assess earthworm populations in 1996 because we did not want to introduce formalin into the enclosures during the year that the lysimeter measurements were being taken.

Lysimeter Installation

Two rectangular, zero-tension lysimeters (43.5 cm length × 31.0 cm width × 5 cm depth), constructed from 1.0-mm-thick aluminium pans filled with pea-sized gravel, were installed 45 cm deep beneath each subplot (enclosure) with increased or decreased earthworm populations ($n = 48$, 2 earthworm treatments × 3 nutrient treatments × 4 replicates). Although corn roots often grow deeper than 45 cm into the soil, an impervious layer at 40–75 cm down limited the rooting depth at this site. During the summer of 1995, a backhoe was used to excavate pits adjacent to the 24 enclosures with increased or decreased earthworm populations and then a cavity was dug beneath the plots at 45-cm depth and the lysimeters were installed 50 cm in front of the edge of the plot. Leachates collected in the pans drained into buried 20-L polyethylene carboys connected to the pans with Teflon tubing. After accumulation of 5 cm of rain in one or more rainfall events, leachates were collected with a vacuum pump into glass collection vessels via a 5-cm-diameter collection tube extending from the carboys to above the soil surface. Ten such events occurred from June to December in 1996. We could not sample earlier in the year because the water table was above the collection vessels as a result of extremely wet conditions. Collected leachate samples were analyzed in the laboratory for inorganic N on a Lachat QuikChem AE flow-injection autoanalyzer using phenate (NH_4) and cadmium reduction–diazotization (NO_3) methods. Concentrations of dissolved organic nitrogen (DON) in leachates were determined as the difference between total NO_3–N in leachates digested by alkaline persulfhate oxidation and the total inorganic N in undigested leachates (D'Elia and others 1977).

Soil Inorganic Nitrogen and Microbial Biomass

We determined concentrations of inorganic soil nitrogen in the upper 15 cm of soil monthly from April through December (except November) and at 15–30 and 30–45 cm in April and October. Soil samples were sieved through a 2-mm mesh sieve and 12 g of field-moist soil were extracted in 50 mL of 2 M KCl. Concentrations of NO_3 and NH_4 in the extracts were determined as described above for soil leachates. Additional samples (0–15 cm) were taken on four dates in 1996 in small subplots in the enclosures to determine soil microbial biomass C using the $CHCl_3$ fumigation–incubation technique (Jenkinson and Powlson 1976; Voroney and Paul 1984).

Crop Productivity and N Uptake

Crop productivity within each plot was measured by harvesting three plants in each of three randomly located areas within each enclosure on October 7, 1996. Corn plants were oven-dried at 60°C. Whole plants were finely ground and analyzed for C and N content on a Carlo–Erba CHN analyzer. Total crop N uptake was determined by multiplying total biomass by tissue N concentration.

Statistical Analysis

A General Linear Model Analysis (GLM; SAS Institute 1990) for a split-plot design was used to compare the effects of the nutrient treatments, earthworm population manipulations, and interactions between nutrient and earthworm treatments on the amount and concentrations of N in water leached through the soil profile, using both repeated measures analysis and data from all dates independently. The interaction between nutrient treatment and block was used as the error term for determining the statistical significance of nutrient treatment effects. The analyses used mean values averaged from the two lysimeters in each field enclosure, and Tukey's HSD mean separation tests were used to determine differences among means. Similarly, data for microbial biomass and total soil C, earthworm populations, and crop productivity were analyzed using an GLM for a split-plot design with nutrient treatment as the main plot effect and earthworm treatment as the split-plot effect.

RESULTS

Earthworm Populations

Earthworm population density in plots with decreased populations was approximately 24% of that in plots with increased populations, and earthworm biomass in plots with decreased populations was 26% and 44% of that in plots with increased populations in April and September, respectively (Table 1). The decrease in earthworm population density and biomass between April and September may have been due to the drier conditions in September which may have induced some earthworms to retreat to deeper soil layers. The data from 1996 are not adequate for accurately describing the earthworm communities at the site because, as stated previously, the hand-sorting method is not adequate for assessing populations of *L. terrestris,* which accounted for a significant portion of earthworm biomass in a previous study at the site (Bohlen and others 1995b). That study used more comprehensive sampling techniques to provide more complete

Table 1. Earthworm Density and Biomass (Ash-Free Dry Mass) as Assessed by Hand Sorting in Plots with Increased and Decreased Earthworm Populations in April and September of 1996

Earthworm population treatment	Earthworm density (No. of individuals m^{-2})		Earthworm biomass (g m^{-2})	
	April 22	Sept. 26	April 22	Sept. 26
Increased	94.6 ± 15.1^{a}	67.5 ± 22.2^{a}	8.4 ± 1.6^{a}	3.6 ± 1.7^{a}
Decreased	21.9 ± 6.3^{b}	16.2 ± 3.8^{b}	2.2 ± 0.9^{b}	1.6 ± 0.5^{b}

Means ± S.E.; n = 12. These data do not represent total earthworm density and biomass in the plots because the hand-sorting method is not adequate for sampling adult individuals of L. terrestris, *which is the largest earthworm species at the site and accounts for the majority of earthworm biomass in the plots (see text for explanation). Values within a column followed by a different letter are significantly different (n = 4, p < 0.05).*

data on earthworm populations in the plots. We summarize that data briefly below to compliment the less complete data from 1996.

In 1993, earthworm populations in field enclosures with decreased populations had 78% fewer earthworms and 73% less earthworm biomass than in plots with increased populations, which is very similar to the data from the spring of 1996 (see details in Bohlen and others 1995b). Of the four species present at the site (*Aporrectodea trapezoides* Dùges, *Aporrectodea tuberculata* Eisen, *Lumbricus terrestris* L., and *L. rubellus* Hoffmeister), *L. terrestris* was the only anecic species present and contributed a significant proportion to total earthworm biomass, especially in plots with increased populations (44% and 61% of total earthworm biomass in plots with decreased and increased populations, respectively). The distribution of *L. terrestris* is important from the perspective of leaching because individuals of this species form deep (greater than 1 m) permanent vertical burrows that can act as preferential flow pathways through the soil (Edwards and others 1989). The other major species at the site, *A. tuberculata,* was an endogeic species that forms a network of subvertical burrows mainly in the upper 20 cm of soil (Edwards and Bohlen 1996). Overall population density and biomass were greater in 1993 than in 1996, a difference that cannot be accounted for completely by the difference in sampling methods. Earthworm populations were even greater in 1991, when the experiment was started, indicating that earthworm populations declined during the experiment.

Transport of Water and Nitrogen

Leachate volumes, averaged across all nitrogen treatments, were consistently and significantly greater in plots with increased earthworm populations than in plots with the decreased populations ($p < 0.0001$). Often the volume of leachates collected from plots with increased populations was twice that from plots with decreased populations (Figure 1 and Table 2).

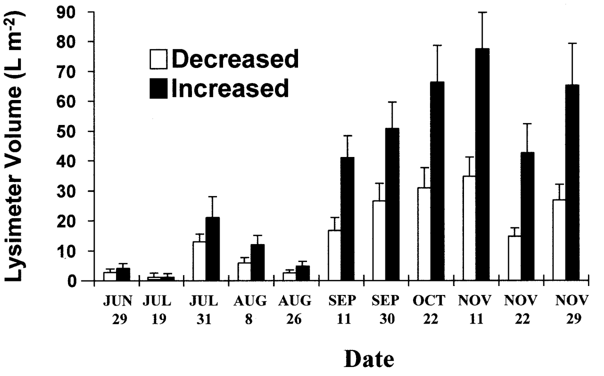

Figure 1. Volume of soil leachates collected from field plots with decreased (open bars) or increased (shaded bars) earthworm population. Values are means ± SE per earthworm enclosure on each of 11 sampling dates (n = 12).

Nitrate concentrations in soil leachates were much greater in plots treated with long-term application of inorganic fertilizer than in plots treated with the legume–grass cover crop mixture or straw–pack dairy cow manure (p = 0.006), and were lowest in the manure-based system. Earthworm population treatment did not significantly influence concentrations of NO_3–N in the leachates (Figure 2, $p = 0.22$). On a single date in August, the NO_3–N concentration in the plots with decreased populations was significantly greater than in the plots with increased populations. There were no significant interactive effects of earthworm population and nitrogen input treatments on concentrations of nitrate. Ammonium–N was not detected in any of the leachate samples.

Concentrations of DON in leachates were significantly greater in inorganically fertilized plots than

Table 2. Results from Repeated-Measures ANOVA Showing *p* Values for the Influence of Fertilizer and Earthworm Population Treatments on Total Leachate Volume and Average Concentrations of Nitrate (NO_3^-), Dissolved Organic Nitrogen (DON), and Total Dissolved Nitrogen in Soil Leachates Collected from Field Plots in 1996

	Probability of *F*			
Source of variation	Volume	NO_3–N (mg L^{-1})	DON (mg L^{-1})	Total nitrogen (mg L^{-1})
Block	<0.0001	0.057	0.205	0.006
Nitrogen treatment	0.329	0.006	0.088	0.002
Earthworm treatment	<0.001	0.220	0.489	0.543
Block × earthworm	0.035	0.572	0.183	0.164
Nitrogen × earthworm	0.507	0.628	0.022	0.334

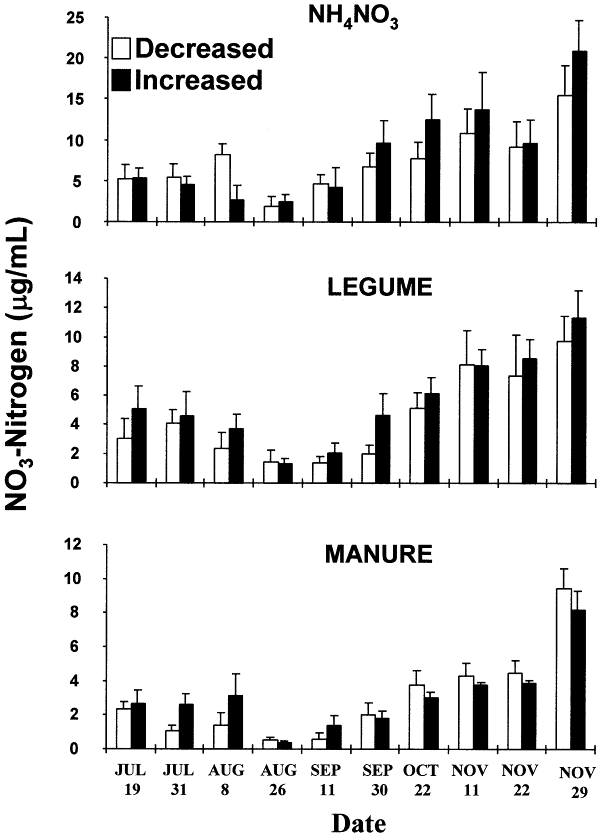

Figure 2. Concentration of nitrate in soil leachates collected from field plots with decreased (open bars) or increased (filled bars) earthworm population in plots treated annually for 7 years with inorganic fertilizer (top panel), legume–grass mixture (middle panel), or cow manure (bottom panel). Values are means ± SE per earthworm enclosure on each of 10 sampling dates (*n* = 4). Note different scales of *y* axes.

in organically fertilized plots. Furthermore, there was a significant interaction between the nitrogen input and earthworm population treatments, in which earthworms had a larger effect on increasing DON concentrations in inorganically fertilized plots than in organically fertilized plots (Table 2 and Figure 3, $p = 0.022$). As was the case with NO_3–N, DON concentrations in leachates were greatest in the inorganic fertilized plots during the fall, which was the period during which earthworms had the greatest influence on increasing DON concentrations in those plots (Figure 3).

Total volume-weighted flux of N to groundwater for the period of measurement was much greater in plots with increased earthworm populations than in plots with decreased populations, due mainly to greater leachate volume in plots with increased earthworm populations (Table 3 and Figure 4). The total flux of nitrogen in soil leachates was 2.5-fold greater in plots with increased earthworm populations (33.9 kg ha^{-1} $year^{-1}$) than in those with decreased populations (13.5 kg ha^{-1} $year^{-1}$), and the contribution of NO_3–N to this flux was much greater than the contribution of DON. Total N leached in the NH_4 NO_3, legume–grass and manure treatments was, respectively, about 2.5-, 3-, and 2-fold greater in plots with increased earthworm populations than in plots with decreased populations. There was a trend toward a significant interaction between earthworm and nutrient treatments on total N flux in which the effect of increased earthworm populations on increasing total N flux was greater in the inorganically fertilized treatment (20.37 kg ha^{-1} $year^{-1}$ in the decreased earthworm treatments and 49.71 kg ha^{-1} $year^{-1}$ in the increased earthworm treatments) than in the legume–grass treatment (10.42 kg ha^{-1} $year^{-1}$ in the decreased earthworm treatments and 31.42 kg ha^{-1} $year^{-1}$ in the increased earthworm treatments) and was lowest in the manure-based system (10.75 kg ha^{-1} $year^{-1}$ in the decreased earthworm treat-

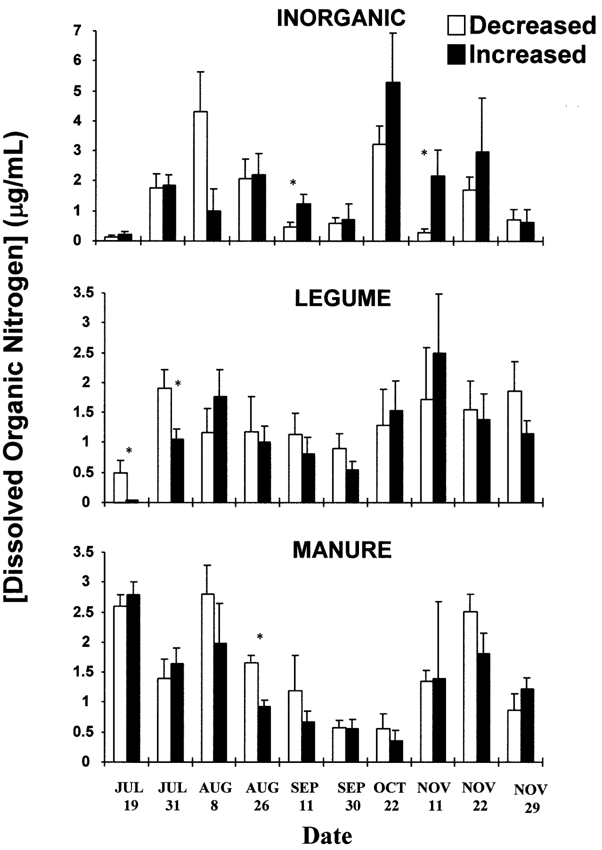

Figure 3. Concentration of dissolved organic nitrogen (DON) in soil leachates collected from field plots with decreased (open bars) or increased (filled bars) earthworm populations and that were treated annually for 7 years with inorganic fertilizer (top panel), legume–grass mixture (middle panel) or cow manure (bottom panel). Values are means ± SE per earthworm enclosure on each of 10 sampling dates ($n = 4$). Note different scales of y axes.

ments and 21.58 kg ha^{-1} year^{-1} in the increased earthworm treatments) ($p = 0.10$ for earthworm × nutrient treatment interaction) (Figure 4). Our measurements excluded the wet spring period prior to June 1996 for reasons explained previously, and thus do not represent cumulative annual losses. Earthworm population density accounted for nearly 50% of the variation in total N flux in the soil leachates (Figure 5).

Soil Nitrate and Microbial Biomass

Soil nitrate concentrations were significantly greater in the inorganically fertilized plots than in the organically fertilized plots at all soil depths when averaged over all dates (ANOVA $p < 0.001$, Table 4). Concentrations of inorganic N in the upper 15 cm of soil in the inorganically fertilized plots were particularly high following application of fertilizer in early summer and subsided to levels comparable to those in the organically fertilized plots after the end of the growing season (November) (Figure 6). It is also possible that inorganic N from organic inputs was released more slowly through mineralization and its release was timed better with crop uptake than was the rapid release of mineral N from inorganic fertilizer. Soil microbial biomass C was significantly higher in the organically fertilized plots than in the inorganically fertilized plots ($p < 0.0001$) and was greater in plots with decreased earthworm population than in plots with increased populations across all nutrient treatments ($p = 0.03$) (Figure 7).

Crop Yield and Nutrient uptake

Crop total N content was significantly greater in inorganically fertilized plots than in organically fertilized plots, and crop yield was significantly greater in plots fertilized with NH_4NO_3 than in plots fertilized with cow manure (Table 5). Plots with increased earthworm populations had significantly lower grain yields than did plots with decreased populations, and there was a trend toward lower total yields in plots with increased earthworm populations ($p = 0.074$) but no significant effect of earthworms on total crop N uptake.

DISCUSSION

Effects of Different Fertilizer Treatments on N Leaching

The much greater N leaching loss from inorganically fertilized systems than from the organically fertilized systems was probably due mainly to the large increase in concentrations of soil nitrate following application of inorganic fertilizer. Nitrate concentrations were greater at all soil depths in the inorganically fertilized plots than in the organically fertilized plots and were especially high in the inorganically fertilized plots immediately following application of fertilizer (Table 4 and Figure 6; Blair and others 1997). Nitrogen leaching losses in inorganically fertilized systems are related to N application rates and cumulative leaching losses can be up to 25% of N applied as fertilizer (Brye and others 2001). Results from tomato (*Lycopersicon lycopersicum*) and corn production systems in northern California showed that greater leaching potential of inorganically fertilized systems was related to greater net N mineralization potential, although organically fertilized systems had a larger pool of po-

Table 3. Results from Repeated-Measures ANOVA Examining the Influence of Fertilizer and Earthworm Population Treatments on Cumulative Nitrate (NO_3^-), Dissolved Organic Nitrogen (DON), and Total Dissolved Nitrogen in Soil Leachates Collected from Field Plots in 1996

	Probability of *F*		
Source of variation	**NO_3–N**	**DON**	**Total nitrogen**
Block	<0.001	<0.001	<0.0001
Nitrogen treatment	0.001	0.027	0.0023
Earthworm treatment	<0.001	<0.001	<0.0001
Block × earthworm	0.0005	0.0003	0.0003
Nitrogen × earthworm	0.145	0.065	0.100

tentially mineralizable N (Poudel and others 2002). Another long-term (12-year) fertilizer experiment showed that increasing levels of manure application decreased NO_3 concentrations throughout the soil profile relative to inorganic fertilizer application or no fertilizer application and this was most likely due to the greater potential for immobilization of N in plots receiving manure (Tong and others 1997). Similarly, in a 15-year study comparing three corn–soybean systems, a legume-based cropping system had reduced nitrogen losses relative to an inorganically fertilized system, and this was attributed to the use of low carbon–nitrogen (C:N) ratio organic residues that significantly increased the retention of soil nitrogen (Drinkwater and others 1998).

In our experiment, the much greater microbial biomass in organically fertilized plots than in inorganically fertilized plots indicates a greater potential for N immobilization in the organically fertilized plots (Figure 7) and greater total soil C in the organically fertilized plots. Average soil C content, determined for all plots, was 2.29, 3.05, and 3.26% in the NH_4NO_3, legume–grass, and manure treatments, respectively ($p = 0.048$). The significantly lower microbial biomass and total soil C in the inorganically fertilized plots signals a lower potential for immobilization of N in the upper soil layer in those plots and greater potential for nitrate leaching, especially when coupled with the higher concentrations of mineral N in those plots. Although we did not directly measure N immobilization in our plots, in a more detailed study of microbial immobilization of N in organic versus inorganic tomato cropping systems, there was significantly greater immobilization of NO_3^- by heterotrophic microbes in the organically fertilized systems (Burger and Jackson 2003).

The drop in inorganic N content over the growing season in the inorganically fertilized plots may have been due to crop N uptake, which totaled 220 kg ha^{-1} in those plots (Table 5). However, significant N leaching loss also occurred during this period, some of which may have come from fertilizer N. The inorganic N concentration in the organically fertilized systems stayed relatively steady over the growing season, indicating that these systems had a pool of potentially mineralizable N (PMN) that provided a steady release of N throughout the growing season to meet crop demand (Burger and Jackson 2003). Despite that steady N release, the organic systems did not provide a sufficient amount of mineral N to support the same level of N uptake as occurred in the inorganic systems, and consequently total crop N uptake in the organic systems was significantly lower (40–60 kg N ha^{-1} lower) than in the inorganic system.

The Role of Earthworms in N Leaching

The much greater N leaching losses from plots with increased earthworm populations, regardless of fertilizer treatment, were due mostly to increased leachate volume, indicating that the effects of earthworms on surface hydrology rather than on N cycling processes had the greatest effect on N leaching. The magnitude of the effects of earthworms on N leaching indicates the potential for earthworms to have a significant effect on the transport of N to ground water in agroecosystems, and possibly even influence the potential environmental risks of these systems. We found that earthworm population density explained nearly 50% of the variability in the total N fluxes in soil leachates (Figure 5).

Earthworms can increase infiltration of water into soil by creating burrows that become pathways of preferential flow under certain environmental conditions (Shipitalo and Edwards 1996). Earthworm channels can range in size from 1 to more than 10 mm in diameter (Lee 1985) and in some cases extend to depths of 2 m (*L. terrestris* burrows; Ehlers 1975). These burrows can influence the in-

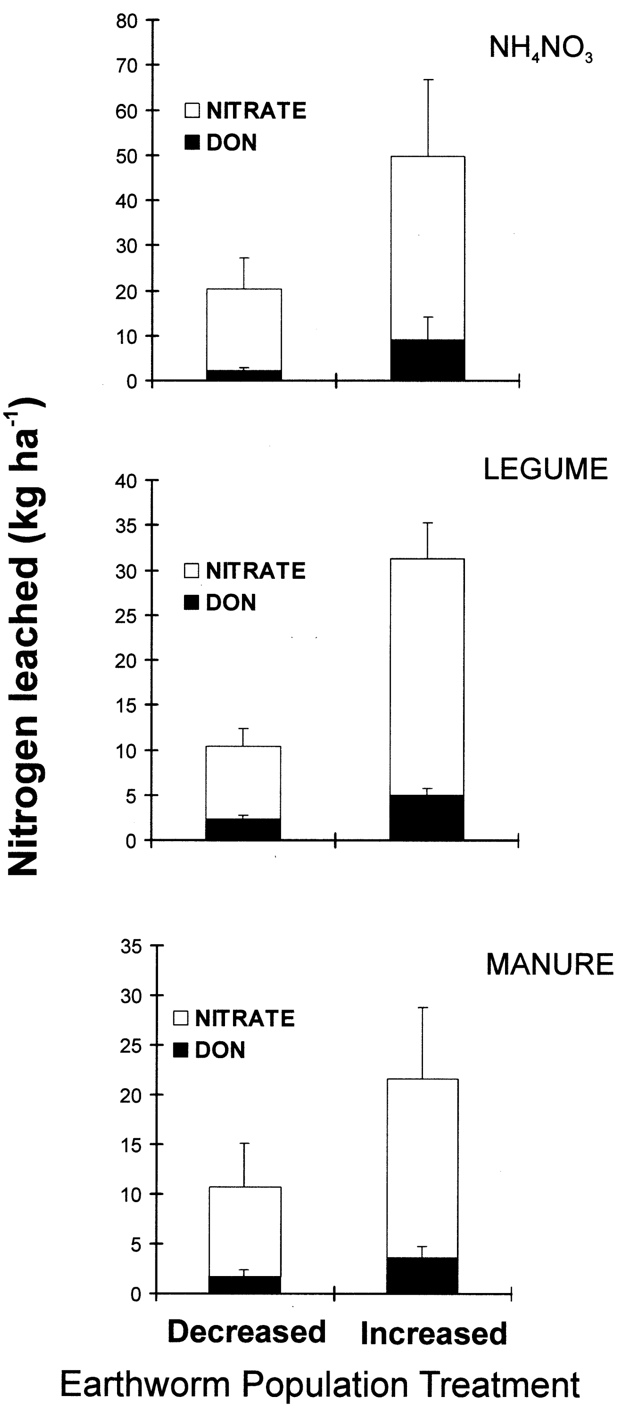

Figure 4. Total dissolved nitrogen in leachates collected from June to December 1996, in plots with increased or decreased earthworm populations that were treated annually for 7 years with inorganic fertilizer (top panel), legume–grass mixture (middle panel), or cow manure (bottom panel). Total losses of dissolved N include inorganic (open bars) and organic (shaded bars) forms of N. Values are means ± SE for cumulative leaching in each plot over 10 sample dates ($n = 4$). Note different scales of y axes.

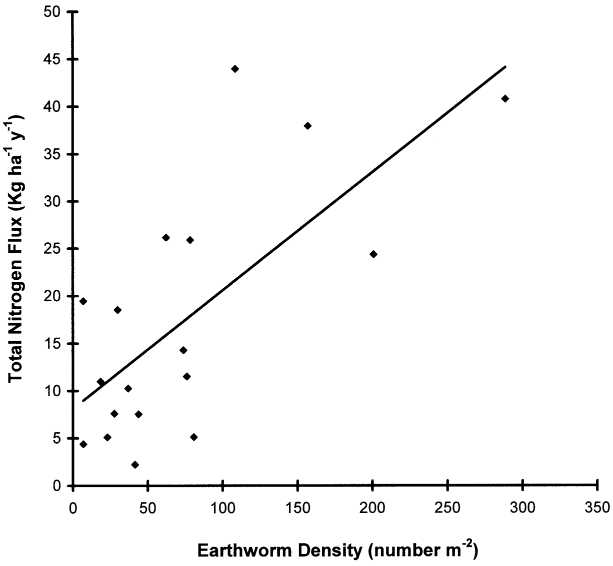

Figure 5. Regression analysis of total leaching N flux versus earthworm population density during the period of measurement.

filtration of water into the soil (Beven and Germann 1982; Germann and others 1984; Joschko and others 1989). In one study it was reported that infiltration rates in soil with higher densities of earthworm-associated macropores were six times greater than in soil with lower densities of such macropores, although the consequences of such increased infiltration for nutrient flux were not determined (Ehlers 1975). The water flowing through earthworm burrows interacts with the burrow lining, which can have elevated N mineralization and N availability relative to the surrounding soil matrix and is also lined with nitrogenous mucus secretions (Edwards and others 1992b; Görres and others 1997). Therefore, water flowing through earthworm burrows may have interacted disproportionately with a small, spatially explicit portion of the soil microenvironment that may have had different N concentrations and dynamics than the surrounding soil.

Inorganic N concentrations in leachates tended to be greater in plots with increased earthworm populations than in plots with decreased populations in the plots fertilized with NH_4NO_3 or the legume–grass mixture, particularly in the fall when leaching losses were greatest. Nitrate concentrations in soil (across all sample dates and depths) also tended to be greater in plots with increased earthworm populations than in plots with decreased populations ($p < 0.09$, Table 2), although these effects were not as clear as those observed previously in the same plots

Table 4. Average Soil Nitrate Concentrations in Field Plots with Increased or Decreased Earthworm Populations and Three Different Long-term (7-year) Fertilizer Treatments[a]

Fertilizer treatment	Soil depth (cm)	N	[KCl extractable NO_3^- (mg L^{-1})] Earthworm population treatment: Increased	Decreased
NH_4NO_3	0–15	28	16.59(3.82)	14.36(3.52)
	15–30	8	8.15(1.03)	7.72(1.81)
	30–45	8	6.03(0.84)	5.68(1.26)
Legume–grass	0–15	28	5.84(0.61)	5.70(0.48)
	15–30	7	4.99(0.74)	4.76(0.39)
	30–45	8	3.32(0.33)	4.72(0.66)
Cow manure	0–15	28	5.68(0.63)	4.96(0.58)
	15–30	8	5.19(0.88)	4.31(0.79)
	30–45	8	3.39(0.62)	2.95(0.57)

[a] *Values are means per earthworm enclosure averaged over all samples dates with standard errors in parentheses.*

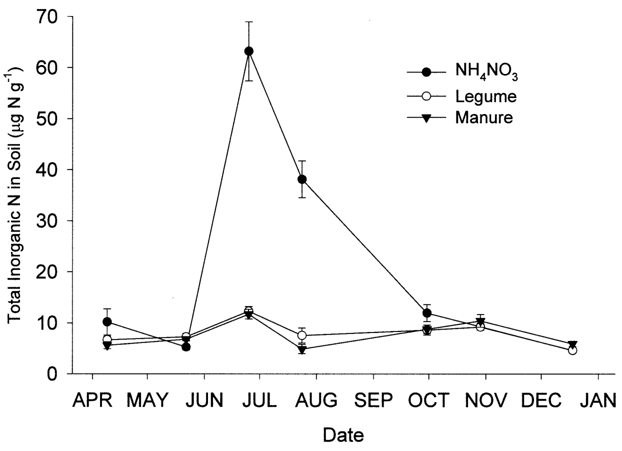

Figure 6. Total inorganic N in the upper 15 cm of soil in plots treated annually for 7 years with inorganic fertilizer (filled circles), legume–grass mixture (open circles), or cow manure (filled triangles). Values are means ± SE for 12 plots. Fertilizers were added to the plots on June 6, 1996.

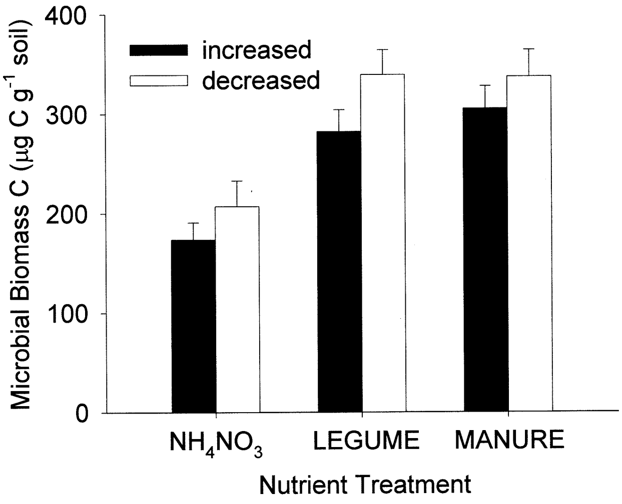

Figure 7. Microbial biomass carbon in plots with decreased (open bars) or increased (filled bars) earthworm populations in plots treated once annually with inorganic fertilizer, legume–grass cover crop, or cow manure. Values are means ± SE for measurements taken on four dates in 1996, $n = 16$. Microbial biomass C was significantly greater in organically than in the inorganically fertilized plots (ANOVA, $p < 0.0001$), and greater in plots with decreased earthworm populations than in plots with increased earthworm populations ($p = 0.03$).

(Blair and others 1997). Furthermore, earthworms decreased microbial biomass which is a key element for immobilizing nitrogen and reducing nitrogen loss in disturbed ecosystems (Vitousek and Matson 1984) (Figure 7). Although decreased microbial biomass does not necessarily translate into greater net N mineralization, results from a microcosm study using soil and earthworms from our study plots showed that earthworms decreased soil microbial biomass and increased inorganic N concentrations (Bohlen and Edwards 1995). Such decreases in microbial biomass with concomitant increases in N mineralization suggest that earthworms can reduce nutrient immobilization and increase net mineralization either by directly consuming microbial biomass or by other indirect mechanisms, for example, by competing with microbes for resources or altering microbial community structure (Lachnicht and Hendrix 2001; Parmelee and others 1998).

The effect of earthworms on increasing the total loss of DON and the concentration of DON of leachates in the inorganically fertilized treatments shows that the organic form of N must be included

Table 5. Grain and Total Crop (Grain + Stalks) Yield and N Content (Means ± SE) in Enclosures with the Three Different Nutrient Treatments and Either Increased or Decreased Earthworm Populations Used in the Lysimeter Studies

Experimental treatments	N	Grain yield (Mg ha^{-1})	Total crop yield (Mg ha^{-1})	Total crop N (kg N ha^{-1})
Nutrient inputs				
NH_4NO_3	4	7.4 ± 0.3^{a}	14.4 ± 0.7^{a}	220.8 ± 15.6^{a}
Legume–grass	4	6.7 ± 0.4^{ab}	12.9 ± 0.7^{ab}	181.4 ± 12.9^{b}
Cow manure	4	6.2 ± 0.4^{b}	12.3 ± 0.7^{b}	161.0 ± 11.7^{b}
Earthworm populations				
Increased	12	6.2 ± 0.3^{b}	14.0 ± 0.6*	178.2 ± 12.0^{ns}
Decreased	12	7.2 ± 0.5^{a}	12.3 ± 0.9	200.6 ± 20.7^{ns}

Different letters with each column signify significant differences between nutrient treatments or between earthworm treatments at the $p < 0.05$ level.
$*p = 0.074$.
ns = not significant: $p = 0.156$.

when considering the potential effects of earthworm on N leaching. Interestingly, Subler and others (1997) showed that addition of deep-burrowing earthworms to inorganically fertilized row-crop agroecosystems in southern Ohio significantly increased short-term leaching losses of N and that most of this increase was due to increased loss of DON. In our study the effects of earthworms on increased N leaching were greater for inoganic N than for organic N and were due mostly to increases in leachate volume, not concentration, but DON still made up a significant part of the earthworm-enhanced N loss.

It is unlikely that inorganic N from any possible increase in dead earthworm tissue in plots with increased populations would have contributed significantly to inorganic soil N or soil leachates in those plots. These plots received an average of approximately 8 kg N ha^{-1} in living earthworm tissue every year that earthworms were added to the plots. This amount of N represented only 4%–5% of total N added to the plots in fertilizer. Furthermore, in 1993 plots with increased populations had nearly 10 additional kg N ha^{-1} bound up in living earthworm tissue than did plots with unmodified populations, indicating that a significant amount of N added in live earthworms was maintained in living tissue and thus could not have contributed measurably to inorganic soil N or leachate N.

If earthworms increased infiltration then they may have decreased overland flow or surface runoff. Earthworms were shown to decrease surface runoff in a large-scale study in New Zealand in which they were chemically eliminated from pastures leading to an increase in the amount of runoff from the pastures (Sharpley and others 1979). Thus, in nutrient-rich agricultural environments, earthworms may, in some cases, shift the balance of hydrologic nutrient loss from surface runoff to leaching loss. Runoff from the plots was not quantified during the period of measurement, so we do not know whether our earthworm population treatments influenced surface runoff in our plots.

In addition to the effects of earthworm population density on N flux, earthworm community structure may also have contributed to differences in N flux between plots with increased and decreased earthworm populations. *Lumbricus terrestis* was significantly more abundant in plots with increased earthworm populations than in plots with decreased earthworm populations (Bohlen and others 1995b). Burrows of this anecic earthworm species have been implicated in preferential flow of water (Shipitalo and Edwards 1996) and short-term increases in water and N leaching in corn agroecosystems (Subler and others 1997). Increased earthworm populations in our experimental plots were linked to a greater density of continuous macropores capable of transferring water from the soil surface into the soil profile (Lachnicht and others 1997). Thus, the burrowing activities of a single species, *L. terrestris,* may have contributed disproportionately to the increase in water and N flux that we observed in plots with increased earthworm populations. However, it is difficult to separate the effect of species composition from that of earthworm density on N flux because composition and density covaried with one another in our experiment (Bohlen and others 1995b).

The overall decline of earthworm populations from 1991 to 1996 may have been due to the imposition of a system of continuous corn production.

The study site was used for alfalfa production for 3 years prior to the start of the experiment and then was put into continuous corn production with annual tillage. Earthworm populations can reach a high density in perennial cropping systems, such as alfalfa, but generally decline in response to continuous row-cropping systems with tillage, although they can reach high densities in no-till row-crop systems (Edwards and Bohlen 1996). The range of earthworm population densities encountered among the different earthworm population treatments was comparable to the range of population densities that might be encountered under actual agricultural management scenarios in the region (Bohlen and others 1995a). For example, in a comparison of earthworm population in long-term, experimental, row-cropped watersheds at another location in Ohio (40°22'N, 81°48'W), earthworm population densities varied from 10 to over 350 earthworms m^{-2} with *L. terrestris* comprising from 0 to over 50% of earthworm biomass (Bohlen and others 1995a).

Interactions Between Earthworms and Fertilizer Treatments

There was a trend toward greater losses of N in response to increased earthworm populations in the inorganically fertilized treatment than in the organically fertilized treatments, especially for DON (Table 3, earthworm × nutrient interaction, $p = 0.065$ and 0.10 for DON and total N flux, respectively). The trend for greater effects of earthworms on leaching of DON in inorganically fertilized than in organically fertilized systems is consistent with the greater effect of earthworms on DON concentrations in the inoganically fertililized system (Table 2). It is possible that the potential for N immobilization was lower in the inorganically fertilized plots because of lower microbial biomass (Figure 7) and lower overall soil C content when compared with the organically fertilized plot. Under these more C-limited conditions in the inorganically fertilized plots, any potential increase in N turnover or mineralization due to earthworm activity may have caused greater mobilization of N than would have occurred in organically fertilized plots where N immobilization potential was likely greater than in the inorganically fertilized plots. It is also possible that the lining of earthworm burrows, which contain organic nitrogenous secretions of earthworm mucus and urine, could also have contributed to the increased DON concentrations in leachates in inorganically fertilized plots, when they occurred.

CONCLUSIONS

Overall, our results indicate that earthworms increase leaching of N mainly by increasing the volume of water leaching from the plots. There is some indication that earthworms have the potential to increase N leaching possibly by increasing DON and NO_3^- concentrations at certain times and by decreasing the N immobilization by the soil microbial biomass, but their effect on the volume of water leaching to greater depths in the soil profile accounted for most of the effect on N leaching in our experiment. Although previous studies in grasslands have demonstrated that earthworms can decrease nutrient losses in surface runoff by increasing rates of water infiltration into the soil (Sharpley and others 1979), our data suggest that increased infiltration rates due to earthworms in row-cropped systems may be linked to a potential for greater nutrient loss to groundwater. Such effects would likely differ at locations with different soil types and under different hydrologic conditions. We cannot state for certain whether the nutrients collected by our lysimeters truly represent nutrients that would have been lost from the system. The presence of a hardpan in the soil at 40–75 cm greatly slows downward movement of water to deeper layers at this site, but there were continuous earthworm burrows extending into the hardpan below 45 cm (J. Domínguez personal observation), suggesting that earthworms may have enhanced preferential flow through this layer, which was beyond the rooting zone of the corn plants.

The difference in earthworm population density between plots with increased and decreased populations is comparable to population differences that have been observed under realistic management practices, so our results have relevance to actual management scenarios (Edwards and others 1994; Edwards and Bohlen 1996). Even greater effects of earthworms on N leaching may have been observed if the earthworm reduction treatments had completely eliminated earthworms from the experimental plots. Our plots were tilled annually and there is the possibility that under no-till management, which has become widely used in many areas of midwestern US, there would be an even greater impact of deep-burrowing earthworms on nutrient infiltration and leaching as a result of the persistence of earthworm burrows and lack of soil disturbance (Edwards and others 1989).

Earthworms are generally considered beneficial to soil fertility and to enhance soil quality, but it is possible that they may intensify the nutrient leaching problems associated with short rotation crop production that is the basis for the vast majority of grain production in the US (Keeney and Muller 2000). Efforts to decrease contamination of surface and groundwater by excess N will benefit from a better understanding of interactions among soil fauna, nutrient inputs, soil organic matter, and other physical and biological factors that influence nutrient mineralization, leaching, and surface hydrology.

ACKNOWLEDGEMENTS

We thank Mike Allen for helping install lysimeters and collecting leachate samples and Dave McCartney for helping with field work and providing data on crop productivity. We thank Clive Edwards and Peter Groffman for their leadership and support throughout the project. We owe a debt of gratitude to John Blair for his role as the principal leader in establishing the long-term field plots. Jorge Domínguez was supported by a postdoctoral grant from the Fundación Entorno (Spain). This project was supported by grants from the National Science Foundation (DEB 9020461 and 9419727).

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