

# Effects of Exotic Earthworms on Soil Phosphorus Cycling in Two Broadleaf Temperate Forests

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## ABSTRACT

We compared the biogeochemical cycling of phosphorus (P) in northern hardwood forest plots invaded by exotic earthworms versus adjacent uninvaded reference plots. In three of the six pairs of plots, earthworm invasion resulted in significantly more total P in the upper 12 cm of soil. The finding of increased amounts of unavailable and occluded inorganic P forms in the invaded plots suggests that earthworm activity mobilized unweathered soil particles from deeper layers of the soil, increasing the stocks of total P in surface soils. In two pairs of plots, the earthworm-invaded soils had less total P than the reference soils. In these plots, earthworm activity resulted in augmented rates of P cycling and alteration of the physical structure of the soil that increased loss of P in leaching water, reducing the total amount of P. We hypothesize that the different effects of earthworm invasion on the soil P cycle

result from unique characteristics of the ecological groups of earthworms dominating each site. The invaded plots with increased total P were dominated by the anecic species *Lumbricus terrestris*, a large earthworm that constructs deep, vertical burrows and is effective at moving soil materials from and to deeper layers of the profile. In contrast, the earthworm-invaded plots where the total P in the surface soil decreased were dominated by the epigeic species *L. rubellus*, which feeds and lives in the upper organic layers of the soil. In these plots, earthworms significantly increased the amount of readily exchangeable P in the soil, increasing the loss of this element in leaching water.

**Key words:** exotic earthworms; invasions; phosphorus cycle; northern hardwood forests.

## INTRODUCTION

In many northern forests with well-developed forest floor horizons, adequate phosphorus (P) nutrition depends on the uptake of this element from the forest floor (Paré and Bernier 1989a, 1989b). Any mobile form of P that leaches from this layer to the mineral horizons is likely to be retained in unavailable forms by the high capacity for P fixation that

usually characterizes mineral soils. As a result, plants in these forests accumulate most of their fine roots in the forest floor, where as much as 80% of their annual P requirement is provided through the mineralization of organic forms of P (Wood and others 1984; Yanai 1992). Thus, P cycling in these forests is biologically controlled in the forest floor by a tight coupling of P mineralization and P uptake and chemically controlled in deeper mineral layers by the P fixation capacity and the rates of weathering of primary minerals (Wood and others 1984). Disruption of forest floor horizons by physical mixing with mineral soil, as can occur when earth-

Received 13 February 2002; accepted 19 December 2002; published online 12 January 2004.

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worms invade forest soils, would be expected to profoundly alter the cycling and availability of P in these forest ecosystems. In this paper, we explore the effects of the activity of introduced earthworms (Annelida; Oligochaeta) on the pools and cycling of soil P in two deciduous broadleaf forests in New York State, USA.

The effects of introduced exotic earthworms in temperate forest soils that have developed in their absence have been analyzed in some locations. In general, earthworm-invaded sites showed a decrease in soil organic matter storage and, at least in laboratory experiments, increased mobilization and leaching of nitrogen (N) and P from the soil (Haimi and Huhta 1990; Haimi and Boucelham 1991; Alban and Berry 1994; Scheu and Parkinson 1994a, 1994b).

Earthworm invasion usually results in the rapid elimination of the forest floor, a reduction of the bulk density, and increased microbial activity in the upper mineral soil horizons. The uniformity of these changes across different sites suggests that invasion in temperate forests with a well-developed forest floor should alter the cycling of P and other nutrients in a dramatic but consistent way. For example, Paré and Bernier (1989a, 1989b) studied 10 sugar maple stands distributed over a 9,000-km<sup>2</sup> region in the Quebec Appalachians. Across this large area, all the stands invaded by earthworms showed similar patterns of P distribution and availability that contrasted markedly with the patterns observed in earthworm-free sites. The earthworm-invaded soils had lower concentrations of available P than soils without earthworms. However, a variety of historical, biotic, and environmental factors could influence the effects of earthworm invasion on nutrient cycling in northern forests. Among these factors, the most important are soil properties, land-use history, forest composition, and earthworm species.

The objective of the present study was to examine the responses of P biogeochemistry in northern hardwood forest soils to invasion by exotic earthworms. By quantifying soil P fractions and P leaching in two forest sites with different land-use histories and different earthworm species, we hoped to clarify the mechanisms by which earthworms influence P cycling. Although in the short term, earthworm invasion would be expected to increase available P in the upper soil layers, we hypothesized that leaching losses of P would be increased and that most of the available P would eventually be fixed in unavailable forms as the earthworms incorporated the materials of the forest floor in deeper layers. The long-term result should thus be a decrease in

the pools of available forms of P and a proportional increase in the pools of less available forms of P. Results partially supporting this hypothesis have already been reported (Paré and Bernier 1989a, 1989b). However, these studies did not examine how differences in earthworm communities may have affected observed patterns in soil P fractions and how these differences relate to changes in total P pools and P mobility in the soil profile.

## METHODS

### Study Site and Experimental Procedures

The study areas are described in detail in an accompanying paper (Bohlen and others 2004a). For this report, the following features of the two study areas are the most relevant ones (a) The Arnot Forest is a second-growth forest that was never cleared for agriculture, whereas the forest at Tompkins Farm is a secondary-growth forest that developed on an abandoned agricultural field beginning at least 75 years ago; and (b) although the soils at both sites are classified as acidic Dystrochrepts (pH 4.2–5.0), in the absence of exotic earthworms, the forest floor horizons are much thicker at Arnot Forest (average, 4 cm) than at Tompkins Farm (average, 1.5 cm).

Three sets of paired sampling plots were established at each study area; one plot of each pair had been invaded by exotic earthworms. Each pair of plots was located on similar soils, with similar topographic position and similar forest vegetation structure and composition. The differences in earthworm distribution were probably due to closer proximity to earthworm refugia in wet seepage areas or streams. Based on four soil cores obtained from the walls of soil pits excavated in each plot, we determined that the subsoil characteristics (35 cm), including total P concentrations, were not significantly different at the reference and earthworm-invaded plots.

Six quantitative soil pits (15 × 15 × 12 cm) were excavated at random locations in each plot. The pits were divided into five layers (forest floor and four mineral layers at depths of 0–3, 3–6, 6–9, and 9–12 cm) for further analysis. Coarse fragments larger than 2 mm were removed, and a subsample of the soil was air-dried and stored at 4°C for P analysis. A sample for each depth layer was oven-dried and weighed for determination of bulk density. For the purpose of examining the effects of earthworms on soil pool sizes, it was necessary to apply a correction factor to account for changes in soil bulk density, because any reduction in bulk density would reduce the total mass of soil contained in the 0–12-cm

depth (in effect, the soil has become slightly deeper). This correction was made by assuming that the P concentration in the 9–12-cm depth layer applied to the additional soil necessary to equalize soil masses between the earthworm-invaded and reference plots.

To determine whether earthworm activity has altered P cycling at our study sites, we used Hedley's P fractionation (Hedley and others 1982) on air-dried samples that had been passed through a 2-mm sieve. We followed most of the modifications described by Tiessen and Moir (1993). In this procedure, different P fractions are separated by sequentially treating the soil sample with reagents or using procedures of increasing strength in the following order: ion exchange resin, sodium bicarbonate, sodium hydroxide, hydrochloric acid, and wet digestion with sulfuric acid and hydrogen peroxide. P concentrations were measured in each of the resulting extracts; these P fractions were assumed to have different relative availability to plants. For the sodium bicarbonate and sodium hydroxide fractions, inorganic P was determined in a subsample of the extract after organic matter precipitation, while the total P was extracted by persulfate digestion (Lajtha and others 1999). The organic P in these fractions was estimated as the difference between total P and inorganic P. Total P in the deeper soil samples (35 cm) and in the forest floor samples was determined after extraction by wet digestion with sulfuric acid and hydrogen peroxide (Harmon and Lajtha 1999; Lajtha and others 1999). The P concentration in all the extracts was measured colorimetrically as described by Murphy and Riley (1962).

Foliage was collected for P analysis from the dominant species in the upper canopy in each plot in August 1999. Litterfall was collected in each plot with a network of eight 0.1-m<sup>2</sup> litter traps in fall 1998, 1999, and 2000. Leaf litter was sorted by species from these collections for P analysis. Foliage and leaf litter samples were dried to constant mass at 70°C and ground. Tissue P was measured by plasma spectrometry following digestion in 2N HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>.

Soil solutions were sampled using zero-tension pan lysimeters. In each plot, we positioned two lysimeters at 15- and at 40-cm depth below the soil surface in June 1998. We waited for 2 full growing seasons (summer 1998 and summer 1999) after installation of the lysimeters before initiating our sampling to allow the soil to recover from the disturbance associated with installation. The sampling period at Arnot Forest extended from October 1999 through October 2000; no sampling was possible in

January and February. Tompkins Farm lysimeters were sampled from April 2000 to October 2000.

Samples were returned to the laboratory and filtered (0.45 µm) the same day they were collected and stored at 4°C until they were analyzed for soluble reactive phosphorus (SRP), and total dissolved phosphorus (TP). Dissolved organic phosphorus (DOP) was calculated as the difference between TP and SRP. SRP was analyzed on a ultraviolet/visible light spectrophotometer (uv/vis) using the molybdate colorimetric reaction (Greenberg and others 1992). Persulfate digestion, followed by phosphate analysis, was employed to determine TP (D'Elia and others 1977). We also report the concentration of nitrate, which was analyzed colorimetrically on a Perstorp series 3000 autoanalyzer (Alpkem, Clackamas, OR, USA).

A dye infiltration experiment was conducted at the Arnot Forest study area to obtain qualitative evidence for earthworm effects on patterns of water movement through soils. Six locations were chosen randomly in the proximity of the earthworm and reference plots at Arnot Forest plots 1 and 2, and 30-cm diameter plastic rings were positioned on the soil surface to direct dye addition. We added 1.5 cm of a 2% blue dye solution to each ring. Immediately after infiltration was complete, a straight-edge shovel was used to excavate a cross section through each ring, and the distribution pattern of dye was recorded photographically.

### Statistical Analysis

Data from each study area (Arnot Forest and Tompkins Farm) were analyzed separately. All the soil variables were analyzed by repeated-measures analysis of variance (ANOVA), with two levels of the factor "earthworm treatment" (worm versus reference), three levels of the factor "site," and four repeated measures of the factor "depth" (0–3, 3–6, 6–9, 9–12 cm). All tests were performed on the means of the six samples of each depth/plot, after evaluation of the normality of the data set. In cases where the distribution of the data was not normal, we log-transformed the data and proceeded as described above. Foliage and litterfall P concentrations were compared using two-way ANOVA, with earthworm treatments and site as main factors.

Concentration data for lysimeter samples were analyzed using the mixed linear model (PROC MIXED) routine in SAS (SAS Institute, Cary, NC, USA). This method was chosen because of its ability to handle unbalanced data and to account for the fixed effects of earthworm presence, lysimeter depth, sampling date, and their interactions, as well as the random effects of the three sites within each

study area, replicates within each plot, and their interactions. Significant fixed effects ( $P < 0.05$ ) were evaluated using least square means comparisons with Tukey's adjustment to control the family-wise error rate. Data that were not normally distributed were log-transformed prior to analysis. All figures show nontransformed data and SE. We also repeated all analyses on the total amount of each nutrient in the lysimeter samples (concentration times the volume of water in the lysimeter) to account for possible dilution effects.

## RESULTS

### P Distribution and Abundance in the Reference Plots

In the reference plots, the patterns of soil P distribution among P fractions and soil depths were, with a few notable exceptions, quite similar for the Arnot Forest and Tompkins Farm (Tables 1 and 2). The concentration of P generally increased with increasing strength of the extracting solution, with the lowest concentrations in the resin fraction (1.4% and 1.8% of total P pool at Arnot and Tompkins Farm, respectively). The percentage of total P in labile forms (resin-P, sodium-bicarbonate Pi and -Po) in the upper 12 cm of the soil averaged 16% in the reference plots at both areas. The concentration of resin P and the organic forms in the sodium bicarbonate and sodium hydroxide extracts consistently decreased with depth. In contrast, the HCl-P and occluded P were relatively constant with depth.

The P content of the upper 12 cm of soil was similar in the two study areas ( $88.4 \pm 12.0$  g P/m<sup>2</sup> and  $75.1 \pm 3.7$  g P/m<sup>2</sup> at Arnot and Tompkins Farm, respectively). The clearest difference between the two forests in the reference plots was the much smaller amount of total P stored in the forest floor at Tompkins Farm ( $1.7 \pm 0.3\%$  of P in the upper 12 cm) than at Arnot ( $8.3 \pm 1.8\%$ ). In contrast, the proportion of total P stored in organic forms in the mineral soil at Tompkins Farm ( $32.4 \pm 1.0\%$ ) was much higher than at Arnot ( $14.4 \pm 2.3\%$ ). Finally, the depth gradients in the concentration of various P fractions were generally more pronounced at Arnot Forest than at Tompkins Farm (Tables 1 and 2).

Soil P status in the three no-worm reference plots within each study area was similar with one notable exception: The P status of the reference plot at site 3 at Arnot Forest was different than at sites 1 and 2. There was a much lower content of total P ( $44.2 \pm 10$  g P/m<sup>2</sup>) and a higher proportion of P stored in the forest floor ( $13.7 \pm 3.5\%$ ) in the reference plot

at site 3 at Arnot relative to the other two sites. The relative importance of the P fractions was also different at the reference plot of Arnot site 3, which had lower percentages of total P present as NaOH-Pi and HCl-P and higher percentages of bicarbonate-Pi, NaOH-Po, and occluded P relative to the reference plots at sites 1 and 2. The relative abundance of this latter fraction was especially high, averaging  $54.8 \pm 2\%$  ( $n = 6$ ) of the total P.

### Earthworm Effects on P Distribution and Abundance

Compared with the reference plots, the worm plots at Arnot had significantly higher concentrations of resin-P and NaOH-Po, lower concentrations of bicarbonate-Pi, and NaOH-Pi, and equal concentrations of bicarbonate-Po, HCl-P, and occluded P (Tables 1 and 3a). The general patterns of vertical distribution of the P fractions were similar for the earthworm-invaded and the reference plots at Arnot; that is, significant differences in the concentration of each fraction at different depths did not interact with the presence or absence of earthworms in the plots (Table 3a).

In Arnot Forest, two of the three worm plots (sites 1 and 2) had significantly lower contents of total P (g P/m<sup>2</sup>) in the upper 12 cm of soil than the reference sites (Figure 1 and Table 3). At least part of this reduction in total P was due to the elimination of the forest floor in the worm plots, which contained only 0.6%–0.7% of the total P in the invaded plots as compared to 3%–14% in the reference plots.

The proportional distribution of soil P between unavailable and labile forms was similar in the reference and earthworm-invaded plots at Arnot Forest. In the worm plots, the unavailable fractions of P accounted for 87.2% of the total P, whereas the labile fractions contributed 12.8% (Figure 2). As in the reference plots, NaOH-Pi and occluded P were the most abundant forms of P in the worm plots, accounting respectively for  $27 \pm 2\%$  and  $30 \pm 2\%$  of the total P. However, there were two fractions for which relative abundance was statistically different between the worm and reference plots. The percentage of total P present as resin-P in the worm plots was almost twice as higher as that in the reference plots (Figure 2; and Table 4a). In addition, the earthworm-invaded plots had twice as much P stored in stable organic forms (NaOH-Po) as the reference plots at Arnot. The relative abundance of all the other P fractions was similar in the worm and the reference plots.

Nearly all the statistical tests for earthworm effects on soil P fractions at Arnot showed a signifi-

**Table 1.** Concentration of Different Soil Phosphorus (P) Fractions ( $\mu\text{g P/g soil}$ ), and Bulk Density ( $\text{g/cm}^3$ ) in Earthworm-invaded and Reference Sites in Arnot Forest

Depth (cm)	Resin-P*		Na Bicarbonate-Pi*		Na Bicarbonate-Po		NaOH-Pi*		NaOH-Po*		HCl-P		Occluded P		Bulk Density ( $\text{g/cm}^3$ )*	
	Worm	Ref.	Worm	Ref.	Worm	Ref.	Worm	Ref.	Worm	Ref.	Worm	Ref.	Worm	Ref.	Worm	Ref.
0-3	30.90	21.93	38.42	50.23	85.54	90.65	150.59	229.07	184.82	123.02	102.28	110.82	217.40	190.92	0.60	0.59
	(3.44)	(3.05)	(4.66)	(9.67)	(7.37)	(8.77)	(12.01)	(44.39)	(8.14)	(13.80)	(7.35)	(17.62)	(18.05)	(10.24)	(0.04)	(0.05)
3-6	16.78	11.25	50.06	71.81	55.06	52.83	218.48	361.40	124.48	78.46	107.72	133.67	214.78	202.16	0.65	0.79
	(1.91)	(1.65)	(11.95)	(15.83)	(6.43)	(9.58)	(30.26)	(83.51)	(7.91)	(6.73)	(7.51)	(20.64)	(14.79)	(11.75)	(0.04)	(0.08)
6-9	10.64	11.09	55.67	84.01	38.51	50.10	237.72	426.25	98.17	57.30	100.66	137.75	224.59	241.80	0.75	0.85
	(1.00)	(1.83)	(14.96)	(21.30)	(3.96)	(9.96)	(32.09)	(96.01)	(7.94)	(8.60)	(5.68)	(21.30)	(14.41)	(14.65)	(0.04)	(0.07)
9-12	8.47	10.89	48.72	79.01	34.48	43.51	222.25	406.14	87.21	63.89	85.06	127.39	230.01	255.87	0.81	0.91
	(0.99)	(1.84)	(12.84)	(18.50)	(3.56)	(8.89)	(24.44)	(74.52)	(7.29)	(8.21)	(5.83)	(19.43)	(12.53)	(14.57)	(0.03)	(0.05)

Means and SE ( $n = 18$ ) are given for each depth.

Asterisks indicate fractions that were significantly different ( $P < 0.05$ ) between earthworm-invaded and reference sites.

For additional statistical information on differences between depths, sites, and interactions, see Table 3a.

**Table 2.** Concentration of Different Soil Phosphorus (P) Fractions ( $\mu\text{g P/g soil}$ ), and Bulk Density ( $\text{g/cm}^3$ ) in Earthworm-invaded and Reference Forest Sites of Tompkins Farm

Depth (cm)	Resin-P		Na Bicarbonate-Pi*		Na Bicarbonate-Po		NaOH-Pi*		NaOH-Po		HCl-P*		Occluded P*		Bulk Density ( $\text{g/cm}^3$ )	
	Worm	Ref.	Worm	Ref.	Worm	Ref.	Worm	Ref.	Worm	Ref.	Worm	Ref.	Worm	Ref.	Worm	Ref.
0-3	37.06	36.65	77.30	48.32	126.16	120.52	280.09	186.89	236.80	221.84	140.82	101.89	200.07	193.19	0.77	0.65
	(5.28)	(5.03)	(11.64)	(5.22)	(11.32)	(9.25)	(21.26)	(13.68)	(10.08)	(11.85)	(12.75)	(6.54)	(11.38)	(8.54)	(0.06)	(0.07)
3-6	15.64	15.32	69.67	41.70	93.91	92.61	310.17	216.72	191.86	209.85	139.66	101.03	183.29	201.92	0.73	0.72
	(1.76)	(1.30)	(10.77)	(5.02)	(9.93)	(9.12)	(35.98)	(18.58)	(13.40)	(11.23)	(12.31)	(7.12)	(6.80)	(8.16)	(0.05)	(0.05)
6-9	14.70	11.27	62.61	37.41	79.62	74.33	330.73	232.74	182.30	189.58	140.47	106.66	195.87	214.01	0.71	0.72
	(2.83)	(1.01)	(9.86)	(4.43)	(7.47)	(5.83)	(39.20)	(19.84)	(8.53)	(7.98)	(14.76)	(7.16)	(8.22)	(7.22)	(0.03)	(0.04)
9-12	9.86	9.37	52.54	35.99	64.45	61.73	327.37	238.34	164.61	170.40	143.01	108.35	194.06	213.03	0.74	0.84
	(1.16)	(0.90)	(7.27)	(4.44)	(4.58)	(4.11)	(39.01)	(21.68)	(10.96)	(6.84)	(14.25)	(7.40)	(6.22)	(6.96)	(0.05)	(0.04)

Means and SE ( $n = 18$ ) are given for each depth.

Asterisks indicate fractions that were significantly different ( $P < 0.05$ ) between earthworm-invaded and reference sites. For additional statistical information on differences between depths, sites, and interactions, see Table 3b.

**Table 3.** F Values and Level of Significance for the Analysis of Variance Used to Compare the Concentration of Different Phosphorus Fractions ( $\mu\text{g P/g}$ ), Total P Content ( $\text{g P/m}^2$ ), and Bulk Density ( $\text{g/cm}^3$ ) of the Soil in Earthworm-Invaded and Reference Sites in Two Northern Hardwood Forests in Eastern and Central New York

a. Arnot Forest									
	Resin-P	Bic-Pi	Bic-Po	NaOH-Pi	NaOH-Po	HCl-P	Occluded P	Total P	Bulk Density
Treatment (Worm/Ref.)	39.42**	72.86**	0.56 NS	21.00**	75.07**	1.62 NS	0.01 NS	30.32**	6.44*
Site	9.55*	9.77*	18.03*	363.93**	2.17 NS	2.97**	30.32**	135.28**	11.69*
Depth	35.68**	3.66*	38.29**	30.67**	41.67**	4.37*	8.02*	16.83**	11.72**
Treat. $\times$ Site	5.38*	18.26**	11.50*	114.71**	13.06**	136.67**	4.20*	28.54**	4.79*
Treat. $\times$ Depth	2.75 NS	0.35 NS	0.86 NS	0.78 NS	0.82 NS	1.96 NS	4.61*	6.21*	1.20 NS
b. Tompkins Farm									
	Resin-P	Bic.-Pi	Bic.-Po	NaOH-Pi	NaOH-Po	HCl-P	Occluded P	Total P	Bulk Density
Treatment (Worm/Ref.)	0.06 NS	64.51**	0.55 NS	168.45**	0.38 NS	245.58**	7.53*	12.25*	0.00 NS
Site	98.11**	142.07**	33.42**	364.64**	27.82**	421.64**	37.55**	36.57**	15.12**
Depth	68.27**	6.66*	22.87**	10.25**	15.93**	1.23 NS	1.71 NS	0.28 NS	0.93 NS
Treat. $\times$ Site	7.64*	36.69**	8.14*	90.33**	0.66 NS	94.22**	1.85 NS	10.69*	2.21 NS
Treat. $\times$ Depth	0.27 NS	0.85 NS	0.03 NS	0.06 NS	1.11 NS	0.17 NS	2.04 NS	2.32 NS	1.56 NS

\**p* 0.05; \*\**p* 0.001; NS = not significant. (a) Arnot Forest; (b) Tompkins Farm. (3a)

cant interaction between the presence of earthworms and site. These interactions resulted from the fact that the patterns in site 3 were often opposite those in sites 1 and 2. In particular, the worm plot of site 3 had higher contents of bicarbonate-Pi, bicarbonate-Po, NaOH-Pi, NaOH-Po, and HCl-P than its corresponding reference plot. As a consequence, the total P content of the earthworm-invaded plot of site 3 was actually higher than that of the reference plot.

In comparison with the reference plots, the worm plots at Tompkins Farm had significantly higher concentrations of three fractions (bicarbonate-Pi, NaOH-Pi, HCl-P); equal concentrations of resin-P, bicarbonate-Po, and NaOH-Po; and lower concentrations of occluded P (Tables 2 and 3b).

The concentrations of all the soil fractions except HCl-P and occluded P differed significantly between depths in the worm plots at Tompkins Farm; however, these differences did not show a significant interaction with the presence of earthworms (Table 3b).

In marked contrast to sites 1 and 2 at Arnot Forest, two of the three worm plots at Tompkins Farm had significantly higher amounts of total soil P than the corresponding reference plots (Figure 1). At Tompkins Farm, the earthworms removed the forest floor of the plots, as they did at Arnot Forest; however, because this layer was not as well developed as at Arnot Forest, the amount of P mobilized from this layer was not as high. The percentage of the total P stored in the forest floor was lower in two of three of the worm plots at Tompkins Farm (0.76%–1.12% in the worm plots versus 1.68%–2.64% in the reference plots).

A variety of other small but significant effects of earthworms on soil P fractions were also observed at Tompkins Farm. For example, the relative abundance of the NaOH-Pi fraction was higher in the worm ( $29.1 \pm 1.6\%$  of total P) than the reference plots ( $24.8 \pm 1.0\%$ ); conversely, the NaOH-Po and occluded P were significantly lower in the worm than in the reference plots (Figure 3 and Table 4b).

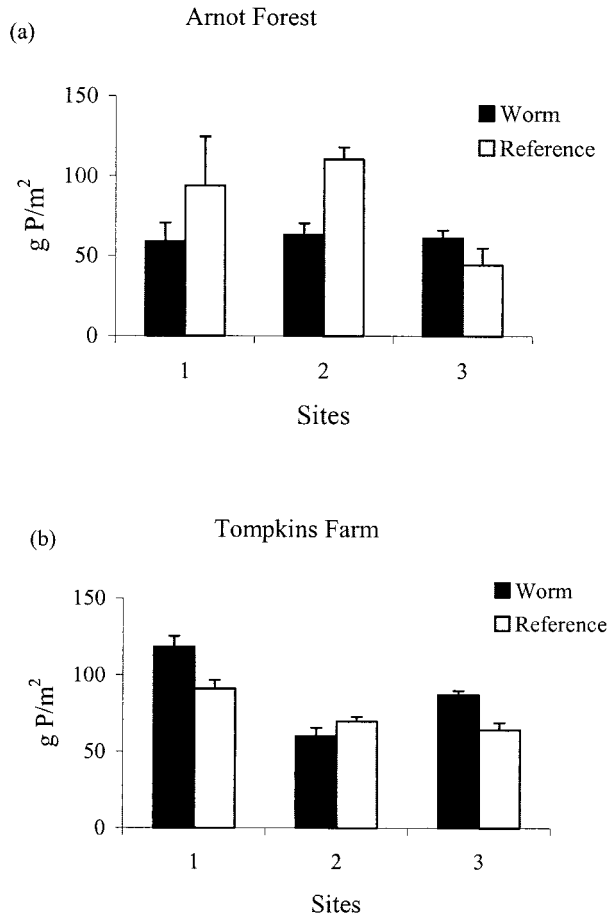


Figure 1. Total soil phosphorus (P) (0–12 cm + forest floor) in three pairs of earthworm-invaded and reference plots in two northern hardwood forests in central (A Arnot Forest) and eastern (B Tompkins Farm) New York. The bars represent means plus SE of 18 soil samples.

The fractions of labile P in the Tompkins Farm worm plots accounted for 15.9% of the total P. The relative importance of resin-P and bicarbonate-Po were similar in the worm and reference plots, whereas the percentage of total P present as bicarbonate-Pi showed a small increase from  $4.5 \pm 0.3\%$  in the reference plots to  $5.9 \pm 0.5\%$  in the worm plots (Figure 3).

Similar to what we found at Arnot, most of the statistical tests showed a significant interaction between the presence of earthworms and the effects of site. At Tompkins Farm, however, these interactions seemed to arise because of subtle differences between the worm and the reference plots at site 2. Notably, the patterns that we found at the earthworm-invaded plot at site 3 at Arnot Forest were qualitatively more similar to those of the Tompkins Farm sites than to those of its neighboring sites at Arnot. At Arnot Forest site 3 and at Tompkins

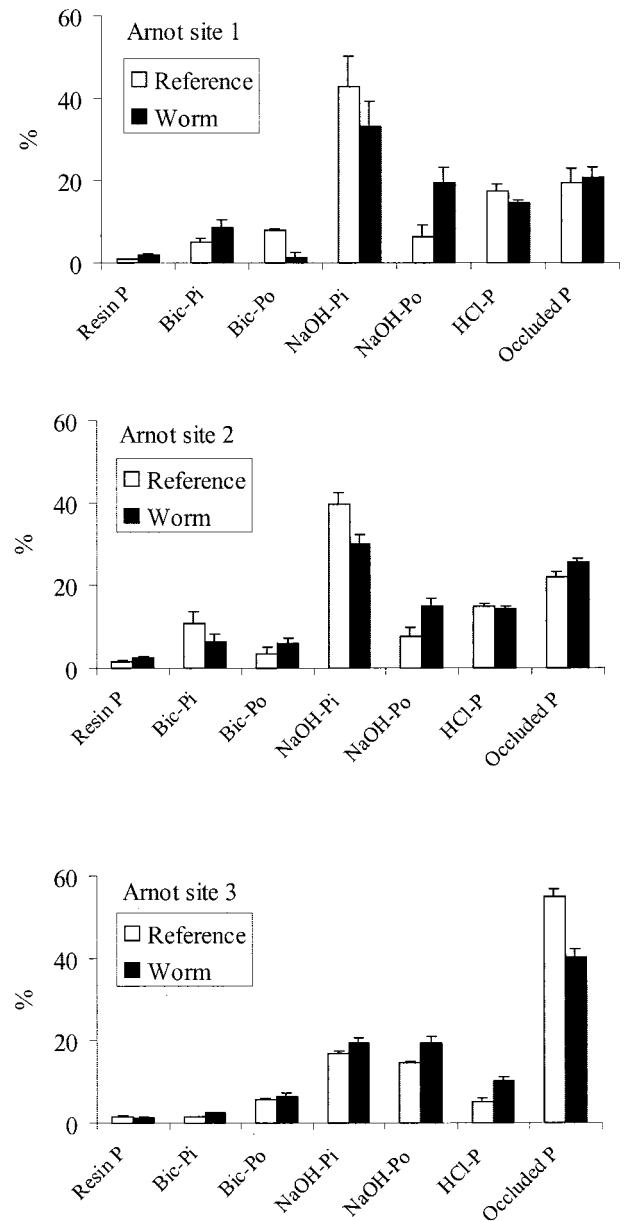


Figure 2. Relative abundance of soil phosphorus (P) fractions in three pairs of earthworm-invaded and reference sites in a northern hardwood forest in central New York (Arnot Forest). The bars represent the mean percentage plus SE ( $n = 18$ ) of each P fraction.

Farm, the worm plots had higher total P and higher amounts of P in NaOH-Pi and HCl-P forms.

#### Foliage and Leaf Litter P Concentrations

Concentrations of P in canopy tree foliage were very similar in the worm and no-worm plots at all the sites (0.13%–0.34% and 0.10%–0.16% across plots and species at Arnot Forest and Tompkins

**Table 4.** F Values and Significance Level of Analysis of Variance Used to Analyze the Effects of Exotic Earthworm Introduction on the Relative Abundance (%) of Different Phosphorus (P) Fractions in Two Northern Hardwood Forests in Eastern and Central New York

a. Arnot Forest							
	√ Resin-P	√ Bic.-Pi	√ Bic.-Po	√ NaOH-Pi	NaOH-Po	HCl-P	Occluded P
Treatment (Worm/Ref.)	12.58**	0.62	1.14	2.11	19.08**	0.50	3.29
Site	9.61**	22.10**	1.47	24.47**	3.39*	33.91**	92.88**
Treat. × Site	7.24**	4.35*	5.54**	2.34	1.39	7.45**	10.92**
b. Tompkins Farm							
	√ Resin-P	Bic.-Pi	√ Bic.-Po	NaOH-Pi	NaOH-Po	HCl-P	Occluded P
Treatment (Worm/Ref.)	0.42	16.81**	1.36	17.24**	10.25**	3.93	15.30**
Site	29.02**	37.19**	0.12	36.72**	29.16**	2.68	31.84**
Treat. × Site	0.86	5.73**	0.47	7.53**	5.13*	0.85	6.61**

The P fractions with a check mark were transformed  $(\ln x + 1)$  prior to the analysis. In each forest, three adjacent earthworm-invaded sites were compared with adjacent reference (worm-free) sites.

Farm, respectively). In contrast, P concentrations and P flux in leaf litterfall were significantly higher at the earthworm-invaded than at the reference plots of both sites. At Arnot, annual P flux in leaf litterfall in the earthworm-invaded plots was  $0.36 \pm 0.02$  g P/m<sup>2</sup>, whereas the corresponding value at reference plots was  $0.31 \pm 0.02$  g P/m<sup>2</sup> ( $F = 51.68$ ,  $P < 0.001$ ). In the same way, the leaf litterfall P flux at the worm plots at Tompkins Farm was higher ( $0.32 \pm 0.04$  g P/m<sup>2</sup>;  $F = 16.24$ ,  $P < 0.001$ ) than at the reference plots ( $0.25 \pm 0.03$  g P/m<sup>2</sup>).

### Soil Solution P Concentrations

Concentrations of SRP in lysimeter solution from 15-cm depth were not significantly different between worm and reference plots at Arnot Forest; however, at 40 cm, SRP concentrations were significantly higher in plots with earthworms ( $P = 0.050$ ) (Table 5). The higher SRP concentrations in worm plots at Arnot was due to significant spikes in SRP concentrations during each autumn ( $P < 0.05$ ) in worm plots but not in reference plots, where SRP concentrations remained consistently low throughout the year (Figure 4). Concentrations of SRP were significantly lower at greater depth in the reference plots ( $P = 0.003$ ) but not in the worm plots (Figure 4). DOP concentrations showed a similar pattern and were significantly higher in the earthworm-invaded than in the reference plots, particularly in autumn ( $P = 0.036$ ), (Figure 5). DOP concentrations in shallow zero-tension lysimeters were sim-

ilar in plots with or without earthworms. All these patterns were consistent across all three plots at Arnot Forest.

Concentrations of SRP in soil solutions at Tompkins Farm were similar to those at Arnot, though much more variable (Table 5). There were no significant differences in SRP concentrations across treatments and depths, but concentrations tended to be higher in late summer/early fall than in April. The DOP data from lysimeters at Tompkins Farm were also highly variable and showed no significant effect of earthworms on DOP concentrations (Table 5).

### Dye Infiltration

Earthworms had an obvious effect on the pattern of dye infiltration in the Arnot Forest plots 1 and 2. In particular, in all locations without earthworms, dye movement was relatively uniform, with most of the soil face showing evidence of dye. In contrast, at three of the six earthworm locations, the dye penetrated deeply (beyond 20-cm depth) along high-flow pathways and was observed to bubble out from earthworm burrows and around soil macro-aggregates in earthworm casts.

## DISCUSSION

The importance of alterations in the biogeochemical cycling of P in response to earthworm invasion of northern temperate forest ecosystems was first sug-

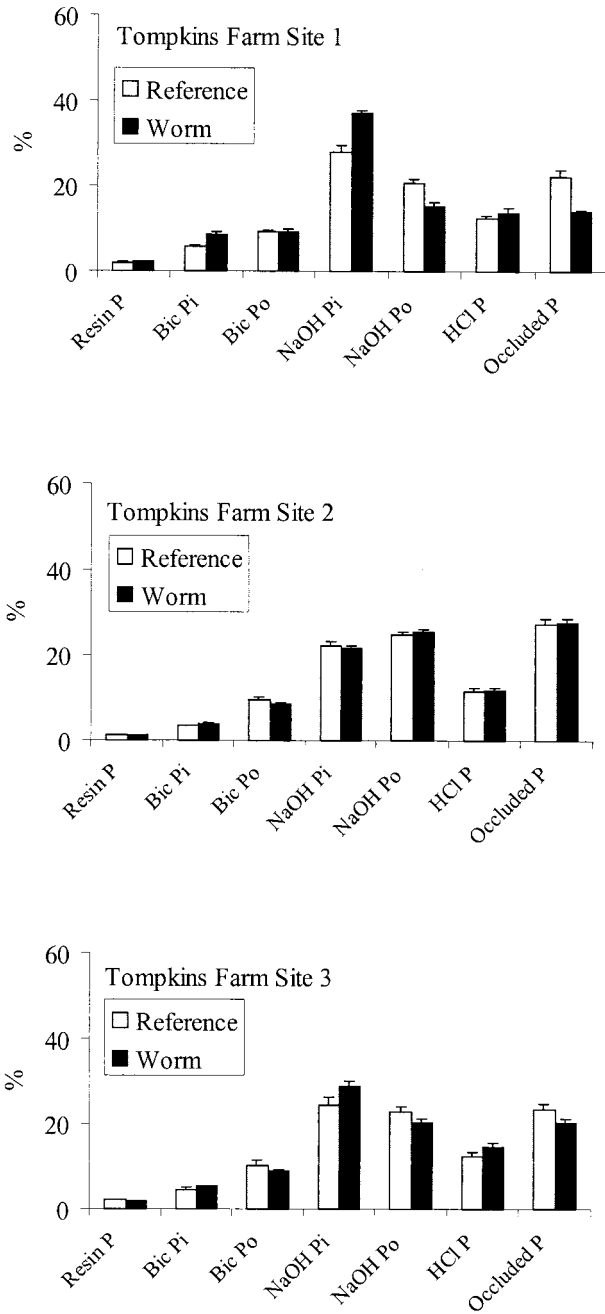


Figure 3. Relative abundance of soil phosphorus (P) fractions in three pairs of earthworm-invaded and reference plots in a northern hardwood forest in eastern New York (Tompkins Farm). The bars represent the mean percentage plus SE ( $n = 18$ ) of each P fraction.

gested by Paré and Bernièr (1989a, 1989b), who concluded that sugar maple decline in Quebec might be related to a P deficiency caused by the earthworm transformation of soils. Although we found no evidence of P deficiency at any of our sites, the profound changes in soil properties that

**Table 5.** Average Concentration of Soluble Reactive Phosphorus (SRP) and Dissolved Organic Phosphorus (DOP) in Soil Solution from Two Depths at Arnot Forest and Tompkins Farm

	SRP	DOP
Arnot Reference		
Shallow (15 cm)	27.7 ± 10.8	8.9 ± 2.8
Deep (40 cm)	4.5 ± 1.9	1.6 ± 1.8
Arnot Earthworm		
Shallow (15 cm)	35.5 ± 21.9	9.7 ± 3.2
Deep (40 cm)	25.0 ± 10.4	8.7 ± 2.8
Tompkins Reference		
Shallow (15 cm)	100.3 ± 87.5	11.4 ± 3.3
Deep (40 cm)	24.8 ± 16.0	23.6 ± 19.9
Tompkins Earthworm		
Shallow (15 cm)	28.5 ± 12.9	8.1 ± 4.5
Deep (40 cm)	35.1 ± 15.7	12.7 ± 4.9

Values are mean ± SE of 9 sampling dates between October 1999 to October 2001 for Arnot and 8 sampling dates between April 2000 and October 2000 for Tompkins Farm.

accompanied earthworm invasion at the Arnot Forest site (Bohlen and others 2004b; Groffman and others 2004; Fisk and others 2004) corresponded with changes in soil P pools and cycling that might precede future disruptions in normal forest dynamics. We interpret our observations in light of the literature on forest P cycling and consider the mechanisms that may account for the observed biogeochemical responses. These interpretations, in turn, provide further insights into the overall effects of earthworms on northern temperate forests observed in our larger study.

### Soil P Pools and Fractions

The concentrations of the different soil P fractions in our study areas fall within the broad range of values reported for other Inceptisols. In those other soils, however, the dominant fractions were HCl-P and occluded P (Schoneau and others 1989; Trasar-Cepeda and others 1991) or NaOH-Po (Cassagne and others 2000), whereas the most important fractions in our sites were NaOH-Pi and, in some layers, occluded P. However, none of the other studies were in temperate broadleaf forests; hence, it appears that the proportions of soil P in different fractions of the Hedley method differ across ecosystems. On the other hand, the proportions of P in plant-available versus unavailable forms that we measured were similar to values reported by Cross and Schlesinger (1995) for a wide variety of soil types (around 14% available forms and 86% unavailable forms).

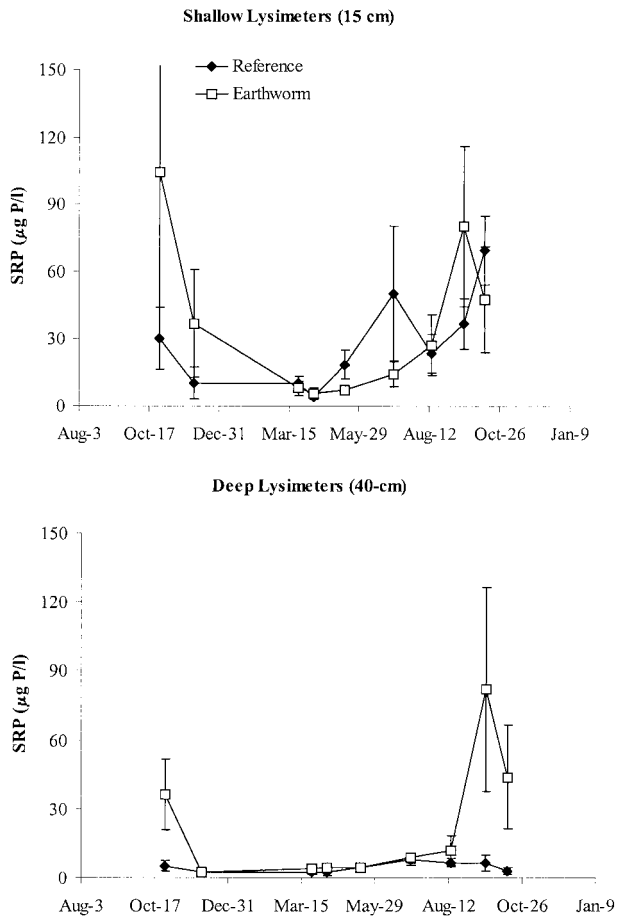


Figure 4. Average soluble reactive phosphorus concentrations ( $\mu\text{g SRP/I}$ ) in zero-tension lysimeters at the Arnot Forest site. Each point represents the mean from the three plots in each treatment. Error bars represent SE.

Our initial hypothesis was that earthworm activity would increase the amount of readily available P in the soil; but that as the earthworms eliminated and incorporated the forest floor into deeper layers, they would increase the amount of nonavailable P forms, because incorporated P would be fixed in Fe and Al hydroxides in the mineral soil. This hypothesis was only partially supported by our measurements. At the worm plots at sites 1 and 2 in Arnot Forest, higher average concentrations and relative abundance of readily available P (resin-P) were observed, together with higher amounts of nonavailable P. However, the increase in nonavailable P was not in the form of P fixed in aluminum (Al) and iron (Fe) hydroxides (NaOH-Pi) as predicted, but rather in the pool of P stored in stable organic matter (NaOH-Po).

The high variation in our results within and between the two study areas indicates that the effects

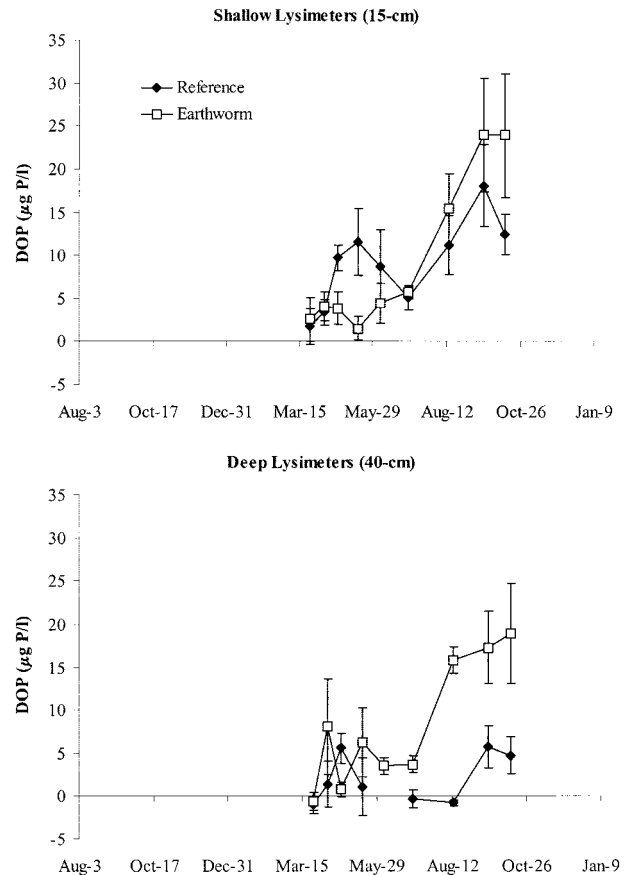


Figure 5. Average dissolved organic phosphorus concentrations ( $\mu\text{g DOP/I}$ ) in zero-tension lysimeters at the Arnot Forest site. Each point represents the mean from the three plots in each treatment. Error bars represent SE.

of earthworm invasion on soil P are more complex than originally hypothesized. This variation suggests that even though the changes in the P cycle induced by exotic earthworms are significant, their magnitude and direction differs depending on other factors. Consequently, generalizations about the large-scale and long-term effects of earthworm introduction cannot necessarily be based on localized measurements at smaller scales.

A comparison of the P content in the worm and reference plots across Arnot Forest and Tompkins Farm reveals two contrasting patterns. In sites 1 and 2 at Arnot Forest, the worm plots had less total P than the reference plots, whereas at the other four sites (Arnot site 3 and all the Tompkins Farm sites) total P pools in the upper soil were higher in the worm plots than in the reference plots. In this section, we discuss the differences in the P fractions that were associated with these contrasting patterns, as well as the factors that may have led to the

divergent effects of earthworm invasion on the P cycle.

In sites 1 and 2 at Arnot Forest, there were two P fractions (resin-P and NaOH-Po) that were present in higher concentrations in the worm plots than in the reference plots. The increase in resin-P in the worm plots could be due to (a) the addition of carbohydrates in the form of earthworm casts, which then compete with phosphate for exchange sites on the soil colloids; (b) increased phosphatase activity in the casts; and (c) the stimulation of microbial activity, which would result in increased mineralization of organic forms of P (Sharpley and Syers 1976; James 1991; Lopez-Hernandez and others 1993; Blair and others 1995; Chapuis and Brossard 1995; Brossard and others 1996). We do not have data to confirm or reject the validity of the first two mechanisms for our plots. However, the third mechanism is supported by observations of increased microbial activity in the surface mineral soil layer of the worm as compared with the reference plots (Groffman and others 2004; Li and others 2002).

The marked increase in the NaOH-Po fraction (organic P stored in stabilized soil organic matter) in the worm plots of Arnot sites 1 and 2 probably can be ascribed to the effect of earthworms on root distribution, or to the direct effect of earthworm casts in stabilizing soil organic matter. On one hand, the earthworm-invaded plots had approximately 50% more fine-root biomass in the upper 9 cm of the mineral soil than the reference plots, and root turnover also may be accelerated by the feeding activity of earthworms (Fisk and others 2004). On the other hand, the casts of many earthworm species facilitate the formation in the soil of stable macroaggregates containing a considerable amount of organic matter that is protected from further breakdown (Shipitalo and Protz 1989; Graham and others 1995). This effect of earthworms seems to be related to the formation of stable organo-mineral complexes in the casts, where the soil particles and organic matter are cemented by internal secretions of the earthworms (Lee and Foster 1991; Tomlin and others 1995; Edwards and Shipitalo 1998). It has been suggested that the formation of stabilized soil aggregates is a key mechanism for stabilizing organic matter in mull soils of temperate deciduous forests (Scheu and Wolters 1991).

Even though the worm plots of sites 1 and 2 at Arnot had greater amounts of available P and stabilized organic P than the reference plots, these plots also had smaller amounts of total P ( $\text{g P/m}^2$ ). This difference resulted from the lack of a forest floor layer, as well as significantly smaller amounts

of P in four of the seven P fractions analyzed (bicarbonate-Pi, bicarbonate-Po, NaOH-Pi, and HCl-P) in the mineral horizons of the worm plots. In the case of the bicarbonate-Pi and NaOH-Pi, both concentration and pool sizes were smaller in the worm plots than in reference plots, whereas for bicarbonate-Po and HCl-P concentrations were similar, but pool sizes were smaller as a result of lower soil bulk densities in the worm plots.

Interestingly, we found a reduction in total P in the worm plots in sites 1 and 2 at Arnot Forest despite the fact that the incorporation of the forest floor must have increased, at least temporarily, the total amount of P in mineral soil. However, when corrected for the soil bulk density, the only P fraction for which pool size increased significantly in the worm plots was the organic P stored in stabilized organic matter (NaOH-Po). What is the mechanism underlying the reduction of total P in the soil invaded by the earthworms?

We believe that this phenomenon can be explained by analyzing the relative abundance of the different P pools. Despite the reductions in the size of the P pools in the worm plots, their relative abundance was practically identical to that of the reference plots (with the exception of the resin-P and NaOH-Po). Assuming that some P leaching occurs with each recycling of P between the organic and inorganic forms of labile P, the smaller size of the P pools in the worm plots and their similar relative abundances suggest that earthworms may have increased the rates of P cycling, thereby increasing the leaching loss of P (Table 5). The mechanisms leading to this acceleration of the P cycle could involve increases in the amount of readily exchangeable P (resin-P), the stimulation of microbial activity in the earthworm-invaded soils, and possibly higher root turnover. This explanation for the reduction of total P in plots invaded by earthworms is also supported by our results showing that the worm plots have higher concentrations of readily exchangeable P (resin-P) and increased microbial activity (Table 1), (Groffman and others 2004; Li and others 2002).

The earthworm-invaded plots at Arnot Forest site 3 and at the Tompkins Farm sites also showed slight increases in the available P fractions; however, the fraction that increased was not the resin-P but the bicarbonate-Pi. This increase in plant-available P could have resulted from the effects of earthworm activity on the exchange complexes of soil colloids. The other P fractions that were different between the worm and reference plots at these sites were the NaOH-Pi and the HCl-P, for which higher concentrations and total amounts of P were observed in

the worm plots. The increases in the pools of these P fractions resulted in higher amounts of total P in these earthworm-invaded plots, in contrast to the lower amount of P in worm plots at Arnot sites 1 and 2.

The increase in NaOH-Pi and HCl-P in the worm plots at these sites is particularly interesting because these two fractions are inorganic forms of P that are fixed to Fe and Al hydroxides (NaOH-Pi) or are part of the structure of primary minerals in the soil (HCl-P). Consequently, our results suggest that earthworm activity in these plots may have affected soil P by two processes: (a) an increase in the availability of Al or Fe hydroxides in the soil, which could be fixing the inorganic P released by earthworm activity; and (b) the mobilization of unweathered primary mineral particles from deeper layers of the soil, resulting in increased amounts of HCl-P in the upper layers of the soil profile.

What factors could explain the striking differences in the responses of soil P fractions to earthworm invasions for these two groups of sites? We propose that the main reason for this contrast is the difference in the earthworm species that populated these sites (Bohlen and others 2004a). In particular, the worm plots of Arnot sites 1 and 2 were dominated by the epi-endogeic earthworm *Lumbricus rubellus* and the endogeic earthworm *Octolasion tyraeum*, which accounted for a much higher proportion of the total earthworm biomass of these plots. In contrast, the earthworm fauna of the worm plot at site 3 in Arnot and the three sites in Tompkins Farm was dominated by the anecic species *Lumbricus terrestris* (Bohlen and others 2004a). Differences between the ecological groups to which these earthworm species belong could significantly influence their respective effects on the P cycle.

The presence of *L. rubellus* at Arnot sites 1 and 2 may account for the loss of total P at these sites, which we contend was a product of increased rates of P cycling and leaching loss induced by earthworms (Table 5). *L. rubellus* has been reported to consume more food and produce more casts per unit of body mass than *L. terrestris* individuals (Shipitalo and others 1988). If the stimulation of the P cycle by earthworm activity depends on the production of casts rich in exchangeable P, then *L. rubellus* could be more effective than *L. terrestris* at increasing the rates of P cycling in the soil. Moreover, it has been shown that *L. rubellus* is more effective than *L. terrestris* at promoting the formation of water-stable soil aggregates (Flegel and others 1998). This could explain the higher amount and proportion of NaOH-Po that we found in the worm plots dominated by *L. rubellus*.

The different burrowing patterns of the dominant earthworm species also could be an important factor. *L. terrestris* is a deep-burrowing species that constructs long channel systems extending from the soil surface to as deep as 1.2 m. Additionally, this species is known to ingest large quantities of mineral particles along with litter fragments and to deposit its casts not only in the deep burrows, but also near or on the soil surface. In contrast, *L. rubellus* is more active throughout the upper mineral soil, ingesting organic rich surface material and depositing casts throughout the upper soil profile. These burrowing and feeding habits suggest that *L. terrestris* could be effective at bringing soil materials from deeper layers up to the top layers of the profile, whereas *L. rubellus* would be more effective at increasing P mineralization, especially in the early stages of invasion. If the materials brought to the soil surface by *L. terrestris* are rich in unweathered soil particles and Al or Fe hydroxides, its presence could explain the higher amounts of NaOH-Pi and HCl-P that we observed in the plots dominated by this species. In this way, *L. terrestris* could be replenishing the total P content of the upper soil layers, thus counteracting the possible loss of P that results from the increased rates of P cycling due to earthworm activities. This mechanism could help to explain why we observed more total P only in worm plots dominated by *L. terrestris*, whereas no increase in total P was seen at Arnot sites 1 and 2, where epi-endogeic and endogeic species dominated.

An additional factor that could contribute to the contrasting effects of earthworm invasion on the P cycle is the land-use history and preinvasion soil properties of the two study areas. Whereas the Arnot Forest study area was in a second-growth forest that had never been cleared for agriculture, the Tompkins Farm sites were postagricultural forests established over the past 60–80 years on abandoned fields. The legacy of past agricultural activities can have long-lasting effects on nutrient cycles (Compton and Boone 2000). Indeed, Bohlen and others (2004b) surmised that land-use history may have contribute to the significantly higher soil solution nitrate concentrations observed at Tompkins Farm versus Arnot Forest. Moreover, land-use history probably influences the species and timing of earthworm invasion at any particular site. Nevertheless, the striking patterns in P pools at Arnot sites 1 and 2 compared with Arnot site 3 and the Tompkins Farm sites argue for an explanation based primarily on earthworm species composition rather than land use per se.

Previous studies examining the effects of introduced earthworms on P cycling in forest ecosystems

have produced divergent results. For example, although Haimi and Einbork (1992) found that the endogeic species *Aporrectodea caliginosa tuberculata* had no effect on the P content of a coniferous forest soil, Haimi and Boucelham (1991) reported that *L. rubellus* slightly increased the P content of a simulated coniferous forest soil. The epigeic earthworm *Dendrobaena octaedra* significantly increased the leaching of P when the different layers of the soil were used separately as substrate for the earthworms (Scheu and Parkinson 1994a, 1994b). However, in laboratory experiments in which all the soil layers were incubated together and in field experiments, *D. octaedra* increased plant growth but not the leaching of P. Scheu and Parkinson concluded that earthworm activity had in fact mobilized a considerable amount of nutrients, but the mobilized P was probably adsorbed on soil mineral particles. Our study seems to partially support this observation, as our findings indicate that earthworm activity could result in the protection of organic P in stable macroaggregates and the increased fixation of inorganic P in Fe and Al hydroxides. However, our results further suggest that this effect depends on the burrowing and feeding habits of the dominant earthworm species.

### Soil Solution P Concentrations and Leaching

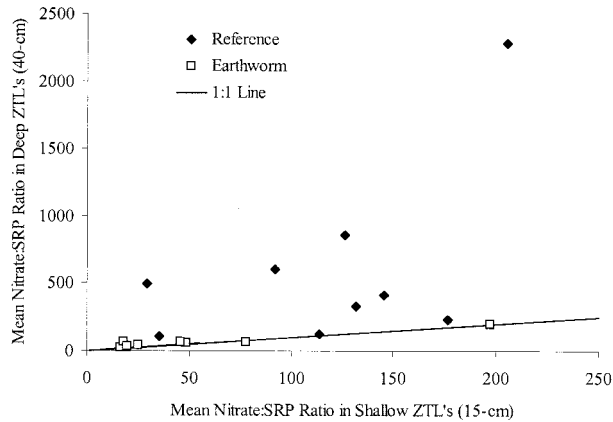
The effects of earthworms on soil solution chemistry can only be understood in the context of their multiple effects on the biological, chemical, and physical characteristics of soil. The initial, and most dramatic, effect is the elimination of the forest floor horizon. We selected our plots based on whether or not the forest floor was present; therefore, all of our worm plots had already passed through this initial phase. After eliminating the forest floor, invasive earthworms can have other persistent effects on forest soils that could influence P leaching. Earthworms deposit casts on the soil surface that dramatically alter soil characteristics. As the earthworms burrow through the soil, depositing casts in deeper layers, they also form macropores capable of conducting water through the soil. Beven and Germann (1982) identified earthworm burrows as potentially significant sites of preferential flow, and several studies from agricultural fields have documented increased water infiltration rates in the presence of earthworms (see, for example, Shipitalo and others 1994, Bouché and Al-Addan 1997).

Finally, earthworm mixing of the soil can significantly alter soil P fractions and microbial activity, with likely effects on P leaching. At the Arnot Forest study area, leaching of SRP was significantly higher

in the presence of earthworms,—a finding that is consistent with previous laboratory studies (Scheu and Parkinson 1994a; Scheu 1997). There are two possible explanations for why SRP concentrations in soil solutions were higher in earthworm than in reference plots at 40-cm depth but not at 15-cm depth (Table 5). The first is that the excess P in the deep lysimeters could be derived from mineralization of organic matter that was mixed into deeper soil layers by earthworms. The second possibility is that the SRP leached from surface horizons penetrated more deeply into the soil in the worm plots. There are two pieces of evidence that support the latter explanation.

First, SRP concentrations in zero-tension lysimeters at both depths in the worm plots were similar and the patterns over time were identical, whereas the patterns in SRP concentrations at both depths were not the same in the reference plots (Figure 4). This result does not preclude the possibility that more P is mineralized at greater depth in the worm plots in the autumn, but it is consistent with SRP removal from solution between 15-cm and 40-cm depth in the reference plots. The possible role of dilution in explaining why SRP concentrations in soil solution did not differ between depths in worm plots can be discounted because our analysis of volume-weighted concentrations produced the same results as the unweighted concentration data (that is, the mass of P collected did not change over depth in the worm plots, but it decreased significantly [ $P = 0.018$ ] over depth in the reference plots). Worm plots also had a higher overall mass of P leached to the lysimeters at 40-cm depth than the reference plots ( $P = 0.006$ ). Therefore, correcting for leachate volume does not change the overall result that more P appeared to leach to a greater depth in the worm plots than in the reference plots.

Second, nitrate concentrations in soil solution at Arnot Forest did not change over depth in either treatment (Bohlen and others 2004b). This result is surprising because N is generally retained in northern hardwood forests and usually decreases in concentration through the soil profile (Christ and others 1995; Lajtha and others, 1995). Previous studies also show rapid uptake and transformation of  $^{15}\text{NO}_3^{\text{N}}$  added to the soil surface (Seely and Lajtha 1997; Nadelhoffer and others 1999). It seems unlikely that  $\text{NO}_3^{\text{N}}$  was transported conservatively in our soils, though it is expected to be more mobile in soil solution than SRP. If SRP was removed from leaching soil water more rapidly than  $\text{NO}_3^{\text{N}}$ , then the  $\text{NO}_3^{\text{N}}:\text{SRP}$  ratio should be greater at depth than nearer the soil surface (Figure 6). This is exactly what was observed in the reference plots at Arnot



**Figure 6.** Ratio of nitrate to soluble reactive phosphorus (SRP) in soil solutions collected from 15-cm and 40-cm depth in reference and earthworm-invaded plots at the Arnot Forest site. Data points represent mean values for 9 collection dates.

Forest, where the mean  $\text{NO}_3^-:\text{SRP}$  ratios for soil solution at 40 cm were significantly greater than the mean ratios from 15 cm. As expected, data points from those reference plots tended to fall above the 1:1 line in Figure 6, presumably because P is rapidly adsorbed on mineral soil surfaces (Frossard and others 1995). However, in the worm plots, the  $\text{NO}_3^-:\text{SRP}$  ratios were not different over depth and fell near the 1:1 line. This result also suggests that little P was removed as the water percolated through the soil in the worm plots and that the removal rate of P relative to N was lower than in the reference plots.

Earthworms could have increased the leaching of P and decreased P sorption during leaching by creating preferential flowpaths through their burrows (Beven and Germann 1982). Such flowpaths have been identified as a mechanism that increases P loss from grassland soils (Hooda and others 1999; Stamm and others 1998; Djodjic and others 1999). We observed strongly preferential movement of dye through the upper 20 cm of soil through earthworm burrows at the Arnot Forest site. Therefore, we conclude that one of the most significant effects of earthworms was to increase preferential flow out of the rooting zone, thereby enabling more P to be lost via leaching. The net result at Arnot Forest plots 1 and 2 is a lower amount of total soil P in plots with earthworms than in reference plots (Figure 1).

In contrast to the Arnot Forest sites, SRP concentrations in soil solutions did not differ significantly between depths or between earthworm and reference plots at the Tompkins Farm sites. No doubt, the differences in earthworm effects between these

sites resulted in large part from the legacy of agricultural land use and the inherently thin forest floor horizons at Tompkins Farm. Although we observed comparable responses in soil P pools at Arnot site 3 and the three Tompkins sites that we tentatively attributed to the difference in earthworm communities, this effect did not translate to a similarity in P leaching responses. That is, there was a consistent pattern of increased SRP concentrations at 40-cm depth during the high-flow season across all three Arnot sites (Figure 4), but it was lacking at Tompkins Farm. Apparently, the dominant effect exerted by thick forest floor layers on the response of these northern temperate forest soils to earthworm invasions masked any more subtle effects of earthworm species composition on P leaching.

## CONCLUSIONS

In conclusion, our study provides only a snapshot of the effects that earthworm invasion may have on P cycling in northern hardwood forests. Our analysis was not designed to control for the effects that other environmental and biotic factors could have on the response of the P cycle to earthworm invasion (for example, earthworm species, time since earthworm introduction, current vegetation, and soil). Consequently, we cannot assess the relative importance of these factors in influencing the P biogeochemical responses in our study sites. However, our results clearly show that earthworm introduction has altered the P cycle in these ecosystems, and they further suggest that both the functional groups of the earthworm species involved and the land-use history of the sites are important determinants of the direction and magnitude of those changes. Experimental approaches are needed to confirm these observations. Specifically, the effects of earthworms of different functional groups should be examined in controlled studies, or by comparing a greater number of sites dominated by different earthworm species. Short-versus long-term effects of earthworm invasion on P cycling could be clarified by comparing newly invaded sites with sites that have been invaded for a long period of time.

## ACKNOWLEDGEMENTS

This research was supported by a grant from the National Science Foundation (DEB-9726869). E. Suárez was also supported by the Ecuadorian Foundation for Science and Technology (FUNDACYT). We thank Isabella Fiorentino, Abe Parker, Emilie Stander, Alan Loreface, Ruth Sherman, and Erik Lilleskov for their assistance during fieldwork and

laboratory analysis. Earlier versions of this paper were greatly improved with comments from Joseph Yavitt, Lars Hedin, Paul Hendrix, and John Pastor.

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