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Earthworm influence on N availability and the growth of *Cecropia schreberiana* in tropical pasture and forest soils

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Summary. This greenhouse study examines the effects of *Pontoscolex corethrurus* on the growth of *Cecropia scheberiana* in forest and pasture soils. Four treatments (0, 2, 20 worms and 0 worms + urea fertilizer) were applied to the soils to test if earthworms affect nitrogen availability, and consequently the growth of *C. scheberiana*. We recorded the number of seedlings, leaves, and height of plants, and measured NH_4^+ and NO_3^- concentrations in water leachates during a six month period. The growth of *C. scheberiana* and nitrogen concentration in leachates differed between pasture and forest soils. Addition of 20 worms per pot doubled nitrate and ammonium concentrations in leachates as compared with the controls. However, earthworms did not affect the germination of *C. scheberiana* in both pasture and forest soils. Although earthworms did enhance nitrogen availability and mineralization, we conclude that nitrogen is not a limiting factor for *Cecropia* growth in either pasture and forest soils.

Key words: *Cecropia scheberiana, Pontoscolex corethrurus*, earthworms, tropical soils, plant growth, N availability

Introduction

Soil organisms are the causal agents of litter decomposition. They influence the rates of mineralization and humification of soil organic matter and ultimately enhance soil fertility (Lee 1985; Lal 1988). In terms of biomass and under different soil conditions, earthworms are often considered the dominant group of soil macrofauna (Fragoso & Lavelle 1992; Blair et al. 1995). Earthworms contribute directly to the nutrient pool in soil solution by adding nitrogenous compounds present in their excreta, mucus and dead tissue, but also affect soil fertility indirectly by tillering, which alters soil physical, chemical and biological properties. This effect of earthworms on soils has been often associated with increased plant productivity, particularly in agroecosystems (e.g., Parmelee & Crossley 1988; Hendrix et al. 1990). However, there is still little evidence of earthworm effects on the growth of forest trees (Lee 1985) under natural and anthropogenically disturbed ecosystems.

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Shifts in land use practices can alter the community structure of both plants and animals (Zou & González 1997). *Cecropia scheberiana* Miq. is an early successional tree species commonly grown in naturally disturbed tropical forests in Puerto Rico. However, it is almost absent in old pastures abandoned fifty years ago (Zimmerman et al. 1995). Zou & González (1997) observed a high earthworm activity in pastures (850 worms/m^2) as compared with the undisturbed forest (50 worms/m^2). Although the recolonization of earthworms on these degraded sites might be beneficial to the restoration of soil fertility, their effects on plant productivity are unknown. Previous studies of earthworms in the humid tropics have shown that their effects on plant growth are variable, but often are associated with changes in soil physical structure and nutrient release (e.g., Pashanasi et al. 1992). In order to understand the plant growth-earthworm interaction, studies using inoculation of different earthworm combinations and plant species are still needed (Edwards & Bohlen 1996). Therefore, this project examines whether earthworm abundance and soils under different land use practices have an effect on the growth of *C. scheberiana*. The main objective is to determine if earthworms affect nitrogen availability, and consequently the growth of *C. scheberiana*.

Materials and Methods

Experimental Design

A greenhouse experiment was carried out during a six month period. A two-way factorial design included two soil types (pasture vs. forest) and three worm densities (0, 2, 20 worms per pot) as treatments. Soils were collected from an active pasture and a mature forest (0–25 cm soil depth) at the town of Sabana (18° 18′ N, 65° 50′ W) in the northeastern Luquillo Mountains of Puerto Rico. Both soils are Oxisols, belonging to the Zarzal series with high clay content (Zou & González 1997). Soils were autoclaved to kill seeds and earthworm cocoons before placing into the greenhouse pots. The abundance of worms used in this experiment were taken as a reasonable approximation of the average population density as in the field conditions for the pasture and forest sites from where the soils were taken. Each treatment was replicated five times, for a total of 30 pots (19 cm diameter × 17 cm tall). In addition to test if N availability is the limiting factor affecting *Cecropia* growth, five pots without earthworms were treated with 1 g of urea (NH_4NO_3) fertilizer every three months for each of the two soil types. All pots were equilibrated in the greenhouse for a month before the initiation of earthworm inoculation and nutrient amendments.

Nutrient amendments started the same day of earthworm inoculation. Earthworms (*Pontoscolex corethurus* Muller) were collected from pasture soils, and allowed to empty their guts for 48 hrs before being placed in the pots. Five seeds of *C. scheberiana* were planted in each of the pots one month after the earthworm inoculation, and this day was recorded as day 0. In the bottom of each pot was a plastic funnel containing a 2-mm mesh screen in the wide end. A plastic tube connected each funnel tip to a water collecting bottle that was previously acid-washed. This method allowed for the collection of water leachates from the pots, and help avoid soil contamination and escaping of earthworms into the collecting bottles. The pots were watered twice a week with tap water, receiving the equivalent of 182 ml precipitation per day. This amount is comparable to what they will normally receive under field conditions (Brown et al. 1983). Leachates were collected weekly in the acid-washed bottles each containing iodine at 10 mg/1 L as a preservative. Nitrate and ammonium concentrations from the water leachates were analyzed on a Lachat QuikChem AE Autoanalyzer. We recorded the number of plants per pot, the height of each plant, number of leaves per plant, and the final dry biomass of leaves, stems and roots of each plant.

Data Analysis

The data analysis was performed by using the average of the measurements resulting from the five seeds planted per pot. Nitrogen availability was interpreted as the amount of nitrate and ammonium concentration in the water leachates. Data were tested for homogeneity of variance by using the Levene's test of equality of error variances, and the skewness. Log-transformations were employed when the data did not meet the assumptions of normality.

To look at the effects of soil type and earthworm abundance on the growth of *C. scheberiana* and N availability, two 2 way-MANOVA analyses were performed by using the plant growth and N availability variables as dependent variables and soil type (pasture vs. forest) and worm abundance (0, 2, 20 worms) as the independent variables (SPSS 7.5 for Win). ANOVA was used to compare mean differences in NH_4^+ and NO_3^- concentrations in pasture and forest soils among treatments for each sampling date. When significant differences were found, Scheffe's test was calculated as an posteriori comparison among means (Sokal & Rohlf 1981). To test if N availability is the limiting factor affecting *Cecropia* growth in pasture and forest soils, the means of plant growth variables in control and urea fertilized pots were compared by a one-way ANOVA.

Results

C. scheberiana seeds germinated after two weeks in the greenhouse pots. There was no soil or earthworm effect on its germination rate. Soil type did have a significant effect on plant growth (Table 1). By the end of the experiment, *Cecropia* seedlings grew taller, and had more leaves per plant in the pasture as compared to the forest soil ($P<0.01$) type. In fact, all seedlings died in the forest soil by the end of the experiment except one individual plant that survived in a pot with 20 worms, whereas about half of the seedlings survived on the pasture soil

Table 1. a) *Cecropia scheberiana* growth as measured by the mean of the final: number of plants per pot, plant height, leaves per plant, dry biomass of leaf, stem and root per pot, and b) N concentration from water leachates in response of soil type (pasture vs. forest), worm abundance (0, 2, 20 worms/pot), and the soil type x worm abundance interaction. Degrees of freedom (df), F and *P* values, and Power for a two-way MANOVA are presented

Source	Dependent variable	df	F	*P*	Power
Soil Type	*a) Plant growth*				
	Number of plants	1	14.97	**0.001**	0.96
	Plant Height	1	13.12	**0.001**	0.93
	Leaves/plant	1	11.16	**0.003**	0.89
	Dry biomass leaf	1	11.61	**0.002**	0.90
	Dry biomass stem	1	6.41	**0.018**	0.68
	Dry biomass root	1	8.59	**0.007**	0.80
	b) N availability				
	NO_3^-	1	9.34	**0.004**	0.86
	NH_4^+	1	553.80	**0.000**	1.00
Worm Abundance	*a) Plant growth*				
	Number of plants	2	0.94	0.40	0.19
	Plant height	2	0.31	0.73	0.09
	Leaves/plant	2	0.10	0.91	0.06
	Dry biomass leaf	2	0.42	0.66	0.11
	Dry biomass stem	2	0.65	0.53	0.15
	Dry biomass root	2	0.17	0.85	0.07
	b) N availability				
	NO_3^-	2	9.83	**0.001**	0.97
	NH_4^+	2	22.60	**0.000**	1.00
Soil Type x Worm Abundance	*a) Plant growth*				
	Number of plants	2	2.36	0.12	0.43
	Plant height	2	0.83	0.45	0.18
	Leaves/plant	2	1.00	0.38	0.20
	Dry biomass leaf	2	0.54	0.59	0.13
	Dry biomass stem	2	0.70	0.51	0.15
	Dry biomass root	2	0.22	0.80	0.08
	b) N availability				
	NO_3^-	2	0.24	0.79	0.08
	NH_4^+	2	7.40	**0.003**	0.91

(Table 2). There was not significant effect of worm abundance ($P > 0.40$) or fertilizer treatment ($P > 0.30$) on *Cecropia* growth in either soil type (Table 1 and 2). The final dry biomass of leaves, stems and roots of *C. scheberiana* were significantly higher in the pasture soil as compared to the forest soil ($P < 0.02$), but a worm or fertilizer effect on the final dry biomass was not observed in either soil type ($P > 0.05$) (Table 1 and 2).

Table 2. *Cecropia scheberiana* growth as measured by the mean (± SE) of the final: number of plants per pot, mean plant height (cm), leaves per plant, and biomass of leaf, stem and root (g) per pot in control and fertilizer treatments in pasture and forest soils. Biomass given as dry weight

Soil Type	*Treatment*	*Number of plants*	*Plant height*	*Leaves/ plant*	*Leaf Biomass*	*Stem Biomass*	*Root Biomass*
Pasture	Control	1.6 ± 0.81	4.3 ± 1.99	3.6 ± 1.50	0.25 ± 0.13	0.09 ± 0.05	0.12 ± 0.07
	2 worms	1.8 ± 0.49	8.5 ± 2.75	4.6 ± 1.30	0.46 ± 0.20	0.26 ± 0.16	0.17 ± 0.09
	20 worms	0.4 ± 0.24	7.3 ± 4.95	3.4 ± 2.23	0.26 ± 0.18	0.11 ± 0.09	0.11 ± 0.09
	Fertilizer	1.8 ± 0.49	2.2 ± 0.49	1.4 ± 0.87	0.10 ± 0.08	0.02 ± 0.02	0.05 ± 0.05
Forest	Control	0	0	0	0	0	0
	2 worms	0	0	0	0	0	0
	20 worms	1.1	1.6	0.2	0.006	0.004	0.018
	Fertilizer	0	0	0	0	0	0

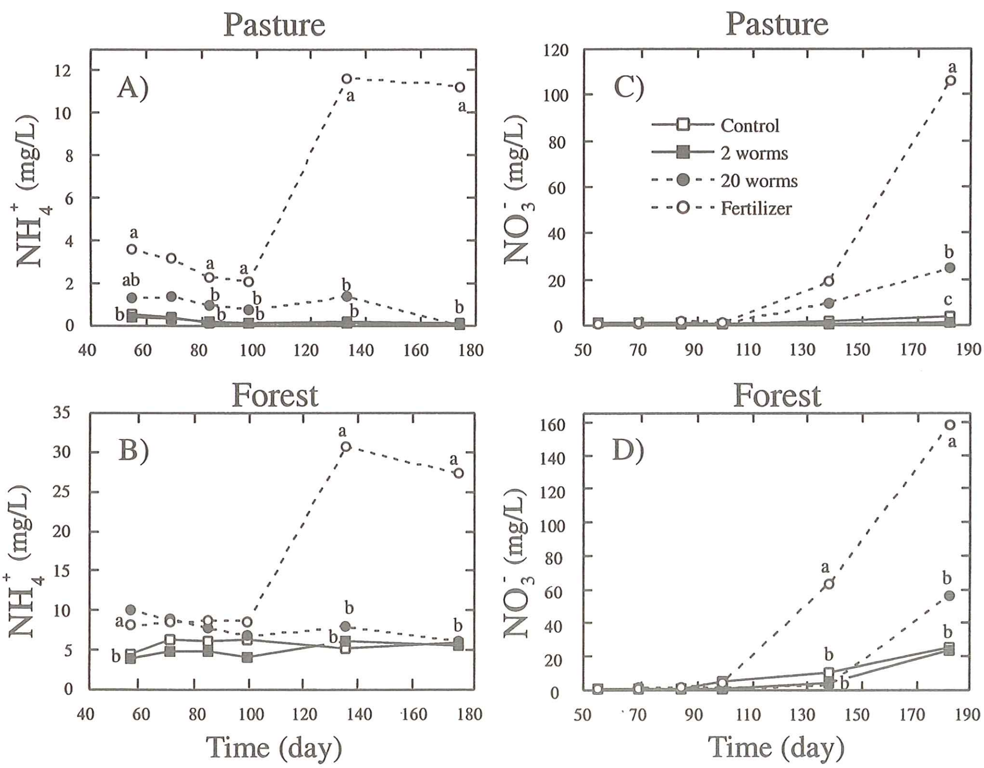

Fig. 1. Ammonium (A, B) and nitrate (C, D) concentration in water leachates as influenced by earthworms, and urea fertilizer in pasture and forest soils. Common letters within a date and forest type indicate no significant difference of treatment on ammonium or nitrate concentration (Scheffe's, ∝ = 0.05)

Soil type had a significant effect on the concentration of NH_4^+ and NO_3^- in water leachates ($P<0.01$) (Table 1). NH_4^+ and NO_3^- concentrations were significantly higher in forest than in pasture soils. Significantly higher amounts of NH_4^+ and NO_3^- leached from urea fertilized pots as compared to the control and worm treated pots (Fig. 1). NO_3^- concentration in water leachates increased throughout time in both soil types (Fig. 1C and D). There was a positive effect of worms on NH_4^+ and NO_3^- concentrations from water leachates ($P<0.001$) (Table 1). By the end of the experiment, NO_3^- concentrations were enhanced by the 20 worms treatment in the pasture soil ($P<0.05$) (Fig. 1C). Cumulative evapotranspiration reached 10 L/pot in both pasture and forest soils, and it did not differed among treatments.

Discussion

C. scheberiana germinated in both soil types, but grew in the pasture soil. These results suggest that differences in biotic and abiotic factors between the pasture and forest soil did not affect *Cecropia* germination, but did affect the survivor rate of young seedlings. Results from this study also seem to indicate that earthworms enhance N availability via nitrification, but nitrogen is not a limiting factor affecting *C. scheberiana* growth in the pasture or the forest soil.

Contrary to our results, it has been shown by Edward & Lofty (1980) with barley, and Stockdill (1982) in pastures that the introduction of earthworms can increase plant production. James & Seastedt (1986), however, found that only plant root growth increased in their experiment. The results found in this experiment are similar to those reported for an experiment that looked at the influence of earthworms on nitrogen fluxes and plant growth in cores taken from variously managed upland pastures (Buse 1990). Buse (1990) found that earthworms increased NO_3^- losses from soil cores taken from unimproved pastures but there was no response in grass growth to increased N availability, suggesting that other soil conditions were limiting plant production.

Pashanasi et al. (1992) reported an enhanced nitrogen mineralization by *Pontoscolex corethrurus*, but observed both a strong increase and a strong inverse response of growth by two tropical fruit tree seedlings after 120 days of inoculation. They inferred that differences in plant growth response were in fact due to physiological differences between the tree species. They discussed how relatively short and thick roots could only exploit a low portion of the soil volume and take little advantage of the increased production of nutrients resulting from earthworm activities. This certainly could be a factor affecting the growth of *C. scheberiana* in both pasture and forest soils. However, we are unable to tease apart direct and indirect effects of worms on nutrient availability as worms did not survive by the end of the experiment. The strong competition of *Cecropia* seedlings with the dense mat of grasses and roots, as well as the increased soil temperature and reduced soil moisture availability due to the lack of a shading canopy in the pasture sites might be some of the factors affecting the regeneration of *Cecropia* on these disturbed tropical pastures.

In conclusion, there is no earthworm effect on the germination of *C. scheberiana* in either pasture and forest soils. Although earthworms enhance nitrogen availability and mineralization, nitrogen is not a limiting factor for *Cecropia* growth in either pasture and forest soils. The mechanism by which *Cecropia scheberiana* is excluded from these abandoned tropical pastures remains to be explored.

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