

Microbial and Microfaunal Community Dynamics in Artificial and *Lumbricus terrestris* (L.) Burrows

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ABSTRACT

Macropore formation and litter incorporation are two results of earthworm [*Lumbricus terrestris* (L.)] activities that can influence trophic dynamics inside burrows. Thus, mesocosms were constructed to examine changes in microbial biomass and microfaunal communities inside artificial compared with earthworm burrows. Four treatments were established: (i) no worms (CTRL); (ii) unlined, artificial burrows (ARTF); (iii) corn (*Zea mays* L.) leaf litter-lined, artificial burrows (LEAF); and (iv) *Lumbricus terrestris* (L.) burrows (WORM). There were no consistent differences in community structures between unlined, artificial burrows and control soils during a 16-wk incubation. In contrast, protozoan numbers were elevated throughout the experiment in LEAF and WORM. A succession of nematode abundances occurred in LEAF, with plant parasitic and Tylenchid nematode numbers peaking at 5 wk, followed by high bacterivorous and fungivorous nematode numbers. In WORM, bacterivorous nematode numbers and active bacterial biomass were elevated for 1 and 3 wk, respectively, before declining. Active fungal biomass increased in WORM, whereas fungivorous nematodes were inhibited in earthworm burrows. While litter incorporation appeared to accelerate the rate of trophic interactions in artificial burrows, the effects of earthworms appeared to transcend that of litter translocation into soil, with earthworms differentially selecting for particular food web dynamics.

EARTHWORMS are well known for increasing soil fertility. Anecic earthworms, in the process of burrowing, accelerate litter decomposition, change pore structure, increase aeration and water infiltration, and accelerate C and N mineralization. Earthworms may also change microbial community composition during gut transit and following excretion in casts and burrow walls (Parle, 1963b; Pedersen and Hendriksen, 1993). For example, bacterial counts increased in earthworm casts compared with “non-cast soil” (Devliegher and Verstraete, 1997). There have been contrasting effects on microbial biomass, with microbial biomass increasing, decreasing, or showing no net change relative to soil unaffected by earthworms (Brown, 1995). The effects of earthworms on the microbial community depend, in part, on the timing of the measurement. For example, Clegg et al. (1995) found that total bacterial counts in burrow and bulk soil were initially no different, but increased through time in casts and remained elevated compared with bulk soil. In contrast, plate counts of a genetically engineered microorganism *Pseudomonas*

fluorescens declined through gut passage of the lumbricid worm *Octolasion cyaneum*, increased significantly 2 d after excretion, and then declined to levels lower than before gut passage (Clegg et al., 1995). In general, regardless of changes in microbial biomass, microbial activity appears to increase in the presence of earthworms.

Some studies have considered the ecological impact of earthworms to be a direct consequence of grazing on microorganisms. For example, reduced microbial biomass N and increased inorganic N and respiration in soil suggested that worms affect nutrient and microbial dynamics by direct grazing of microbes (Bohlen and Edwards, 1995; Zhang et al., 2000). Microbial activity is primarily responsible for degradation of organic materials in soil; however, grazing of microorganisms is known to enhance rates of nutrient mineralization (Woods et al., 1982; Ingham et al., 1985). Earthworms influence biological activity on other levels of food webs as well. Consumption of protozoa is considered to be an important component of the earthworm diet and important in the dispersal of protozoa in cast material (Bamforth, 1988; Bonkowski and Schaefer, 1997). Senapati (1992) observed that plant parasitic nematodes decreased, whereas microbivorous nematodes increased in soil with earthworms; at the same time, litter decomposition increased. Litter decomposition and nutrient release within and outside earthworm burrows depend on the microbial and microfaunal community composition. Previously, we found that nematode numbers increase with a concomitant decrease in microbial biomass and increase in C and N mineralization in soil cores incubated in the presence of *L. terrestris* (Görres et al., 1997). We suggested that earthworms may increase nematode abundance, either through increasing movement of nematodes into the burrows driven by CO₂ gradients and/or through increasing reproduction driven by greater food resource availability, and thereby increase microfaunal grazing of microorganisms in burrow soil. To better understand how earthworms ultimately affect microbial activity and community structure requires a comprehensive look at their impact on different levels of the detrital food web.

The goal of this study was to evaluate the influence of anecic earthworms on microbial and microfaunal communities by measuring active and total bacterial and fungal biomass, as well as abundance and trophic group distribution of protozoa and nematodes. We compared CTRL, ARTF, LEAF, and WORM to determine how macropores with and without litter residue and with

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Published in Soil Sci. Soc. Am. J. 68:116–124 (2004).
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Abbreviations: ARTF, artificial burrows not lined with corn leaf litter; CTRL, control soil; LEAF, artificial burrows lined with corn leaf litter; WORM, *L. terrestris* burrows.

worms affect the decomposer community in drilosphere soil.

Some effects of earthworms may become apparent only after an extended period of time because changes that affect microbial community composition and trophic interactions, such as diffusion of nutrients beyond the burrow walls and development of pore structure in the burrow walls, may occur gradually. Therefore, we conducted laboratory incubations of soil mesocosms for 16 wk to understand faunal and microbial succession as burrows age. In a previous study, we found that community dynamics in burrows may be obscured or even undetected when burrow soil is "diluted" with unaffected bulk soil (Görres et al., 1997). In the present study, we sampled burrow and bulk soil separately.

MATERIALS AND METHODS

Soil Collection

Enfield silt loam soil (coarse-silty over sandy or sandy-skeletal, mixed, active, mesic Typic Dystrudept) with a soil organic matter content of 3.6% was collected in November of 1998 from the Ap and B horizons of an old field in the Peckham Farm Research Area of the University of Rhode Island, Kingston, RI. The soil was stockpiled outside under a black plastic sheet until the end of January. Soil was brought into a greenhouse in late January 1999 and sieved for the next 3 d by passing through a 5-mm metal sieve. Sieved soil was stored uncovered in the greenhouse (total of 4 d) until the start of the experiment.

Establishment of Mesocosms

Experimental mesocosms consisted of 10-cm i.d. and 0.5-m-long white polyvinyl chloride (PVC) cores filled to a depth of 40 cm with sieved soil. The length of the soil column is within the range of burrow lengths observed over which anecic earthworm species line their burrows with litter (Edwards and Bohlen, 1996). Fine fiberglass mesh screening covered the bottom to retain soil and prevent earthworm escape. Cores were filled with soil while intermittently tapping the cores to promote uniform bulk density, resulting in an average bulk density of 1.3 g cm⁻³. Four different treatments (three replicates per treatment) were established: (i) CTRL, (ii) ARTF, (iii) LEAF, and (iv) WORM. All cores received 100 mL (1.25 cm) of tap water after cores were assembled and 2.0 cm of water on Weeks 1, 3, 7, and 10 (equivalent to one-third of the average precipitation received from April to June in RI). Soil moisture content, as determined gravimetrically by drying at 105°C, ranged from 8 to 18%. Soil moisture increased in all treatments until Week 3 and then gradually declined until the end of the incubation. All cores were covered with fiberglass mesh and incubated for a total of 16 wk at 18 to 20°C in the laboratory.

Corn leaves for the mesocosm experiment were collected in October of 1998 from a no-till, organic cornfield at Casey Farm in Saunderstown, RI. The leaves were stored as collected in large, white plastic garbage bags in the dark at 4°C for 3 mo. Corn litter which had visible fungal growth on the surface was cut into approximately 2-cm long pieces on the day the experiment was begun and 5 cm (9 g dry wt.) of corn litter was placed on the soil surface of all treatments. This amount of litter corresponded to 3.42 g C and 0.16 g N per mesocosm. The mean C and N contents of the litter were 38 and 1.8%,

respectively, with a C/N ratio of 21.1. Litter was not replenished to mimic field conditions.

Artificial Burrows

Artificial burrows were prepared using two 9-mm o.d. glass tubes (containing 4-mm diam. glass rods) that were kept in place while the column was packed. Earthworm burrows tend to be 1 to >10 mm in diameter (Lee, 1985). Diameters of burrows made by the larger, anecic earthworms such as *L. terrestris* are generally >4 mm (Lee, 1985). The two glass tubes (45 cm long) were placed directly across from each other, 20 degrees off the vertical in each column to approximately 5 cm from the bottom of the core. After the column was packed, the glass tubes were removed and the glass rods remained inside the 8-mm diameter, artificial burrows as a check to ensure that the artificial burrows did not collapse and to help define the burrows when sampling. To simulate litter in the burrows, we wrapped half a corn leaf (cut lengthwise and excluding the petiole, 1.5 g dry wt.) around the length of the glass rods before packing the column. This corresponded to 0.98 g C and 0.046 g N per mesocosm. The leaf was secured in place along the rods with three loosely positioned nylon cable ties.

Earthworm Burrows

Adult earthworms were purchased from a commercial outlet in North Kingstown, RI 1 d before the start of the experiment and stored at 4°C in the dark. Earthworm treatments consisted of placing three individuals of *L. terrestris* (5.2 g initial fresh weight per individual as measured on 24 individuals) per core on the soil surface. This represented an initial population density of 370 worms m⁻² and was in the range of abundances observed in pasture soils (Lee, 1985). Earthworm mortality was low, but on Weeks 13 and 16, worm density decreased to 290 individuals m⁻². A dead worm was found in two out of three cores (an average of 2.3 individuals per core) on both dates. While we do not know when the worms died, death most likely occurred within 1 wk of sampling since worm tissue is degraded rapidly (Whalen et al., 1999).

Soil Sampling

Three cores from each treatment were sampled destructively after 0, 1, 3, 5, 7, 10, 13, and 16 wk of incubation. Burrow soil was kept separate from bulk soil. We defined burrow soil as that within 5 mm of the macropore wall (regardless of macropore origin). The cores were sectioned (10 cm) and soil was removed from the drilosphere by excavation with a spatula. We combined drilosphere soil from the 10-cm sections within individual cores to obtain a large enough sample to carry out all analyses. Bulk soil was sampled from the CTRL core. Samples were stored at 4°C and processed within 1 wk of the sampling date.

Active and Total Bacteria and Fungi

Active and total bacteria and fungi were analyzed by Soil Foodweb, Inc. (Corvallis, OR). Samples (approximately 20 g moist soil) were shipped on ice by overnight courier. Sampling was timed so that delivery of samples to Soil Foodweb was made within 3 d of sample collection and samples were analyzed within 24 h of receipt. Active bacteria and fungi were counted by the fluorescein diacetate staining (FDA) method (Ingham and Klein, 1982). Total bacterial numbers and fungal hyphae length were determined by phase-contrast microscopy and converted to micrograms of biomass per gram of dry soil

($\mu\text{g biomass g}^{-1}$ dry soil) using the equations in Bottomley (1994) and conversion factors of $0.2 \times 10^{-12} \text{ g } \mu\text{m}^{-3}$ for bacteria and $0.4 \times 10^{-12} \text{ g } \mu\text{m}^{-3}$ for fungi.

Protozoa

Enumeration and identification of protozoa were conducted by Soil Foodweb, Inc. using a most probable number technique (Ingham, 1994). Protozoa were grouped into flagellates, ciliates, and amoebae.

Nematode Abundance and Trophic Group Distribution

Nematodes were extracted for 24 h from soil (20 g moist) using the Baermann funnel technique. Extracts containing nematodes were preserved with formalin. Total numbers of nematodes were determined using a dissecting microscope. Separation of nematodes into trophic groups was based on morphology of stoma and esophagus and known feeding habits of recognizable groups (Parmelee and Alston, 1986; Yeates et al., 1993a), including fungivores and bacterivores. Tylenchids were identified and counted as a separate group because of the uncertainty surrounding their feeding preferences (Yeates et al., 1993a). Data for omnivores and predators were pooled and reported as one trophic group.

Statistical Analyses

Statistical analyses were performed using a one-way analysis of variance (ANOVA). An ANOVA-on-ranks was used to determine statistical differences ($P < 0.05$, unless stated otherwise) among treatments for microfaunal groups when criteria for parametric tests were not met. Microbial biomass and total nematode values for the three burrow treatments were compared with each other and control soil. Pair-wise multiple comparison procedures (either a parametric Student–Newmann–Keuls method or non-parametric Dunn's test) were used to detect significant differences between means. Numbers of nematodes in different trophic groups, as well as numbers of amoebae, ciliates, flagellates, and total protozoa, in LEAF were compared with WORM soils using a t-test. Protozoan abundances were log transformed due to high variance in measurements among replicates.

RESULTS

Microbial Community Dynamics

Active microbial biomass ranged in all treatments from a mean (\pm std. dev.) of $2.66 (\pm 0.33)$ up to $22.54 (\pm 4.67) \mu\text{g g}^{-1}$ soil throughout the incubation and was

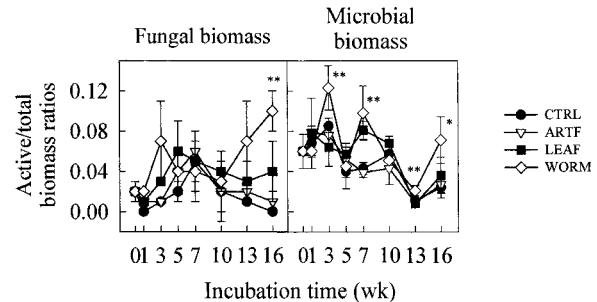


Fig. 1. Active/total fungal biomass and active/total microbial biomass in artificial burrows not lined with corn leaf litter (ARTF); control soil (CTRL); artificial burrows lined with corn leaf litter (LEAF); and *L. terrestris* burrows (WORM) treatments as a function of incubation time. Bars represent one standard deviation ($n = 3$). Asterisks indicate dates that the active/total ratios are significantly higher in WORM soil than in any other treatment (* is for $P < 0.05$, ** is for $P < 0.01$). On Week 7, the active/total microbial biomass ratios for both LEAF and WORM soil were higher than for CTRL and ARTF.

generally highest in WORM soil, with differences being significantly higher than the CTRL on Weeks 3, 5, and 13 (data not shown). Total microbial biomass ranged from $97.07 (\pm 17.27)$ to $383.83 (\pm 182.93) \mu\text{g g}^{-1}$ soil, but showed fewer significant differences among treatments than active microbial biomass. Total microbial biomass was significantly greater in WORM soil than all other treatments on Week 3 and was significantly lower in both LEAF and WORM soil than CTRL and ARTF on Week 7 (data not shown). The greatest number of significant differences between CTRL and WORM were seen in calculations of the active-to-total microbial biomass ratios, which were significantly greater in LEAF and WORM soils than CTRL and ARTF on Week 7 and were greater in WORM soil than all other treatments on Weeks 3, 13, and 16 (Fig. 1).

Active bacterial biomass ranged from 0 to 34% of total bacteria throughout the incubation period in all treatments. Active bacterial biomass in ARTF was significantly lower than in CTRL soil only after 3 and 10 wk of incubation (Table 1). After 5 wk, active bacterial biomass in LEAF was not different from either CTRL or WORM treatments. Active bacterial biomass in WORM soil was about 30% higher than CTRL soil and about 50% higher than ARTF and LEAF at Week 3, but values for all three treatments converged with further incubation (Table 1). Mean values for total bacterial

Table 1. Mean values (\pm standard deviation) for active bacterial biomass in the four treatments[†] as a function of incubation time.

Variable	Time	Treatment				P
		CTRL	ARTF	LEAF	WORM	
	wk					
Active bacterial biomass [‡] , $\mu\text{g g}^{-1}$ soil	1	11.10 (2.03)a§	7.87 (0.54)ab	6.23 (2.03)b	5.63 (1.59)b	0.04
	3	10.80 (0.94)a	6.92 (0.81)b	5.67 (1.72)b	15.67 (0.83)c	<0.001
	5	6.00 (2.62)a	8.67 (1.10)a	8.23 (1.35)a	8.50 (1.59)a	0.44
	7	6.70 (0.14)a	6.07 (0.74)a	7.40 (1.10)ab	9.17 (0.88)b	0.02
	10	13.73 (1.37)a	8.30 (1.24)b	11.07 (0.68)ab	10.30 (1.91)ab	0.03
	13	3.30 (0.96)a	2.13 (0.45)a	1.13 (0.12)a	2.63 (1.11)a	0.11
	16	3.50 (1.14)a	5.37 (1.90)a	4.33 (1.99)a	7.13 (1.32)a	0.22

[†] Control (CTRL); artificial, unlined burrows (ARTF); artificial, litter-lined burrows (LEAF); and *L. terrestris* earthworm burrow (WORM) treatments.

[‡] Initial mean (\pm std. dev.) active soil bacterial biomass was $6.23 (1.29) \mu\text{g g}^{-1}$ soil.

[§] Different letters for a particular incubation time indicate a statistically significant difference ($P < 0.05$) among treatments ($n = 3$).

^{||} P values are given for each one-way ANOVA comparing CTRL, ARTF, LEAF, and WORM treatments.

Table 2. Mean values (\pm standard deviation) for total bacterial biomass in the four treatments[†] as a function of incubation time.

Variable	Time	Treatment				P [‡]
		CTRL	ARTF	LEAF	WORM	
	wk					
Total bacterial biomass, $\mu\text{g g}^{-1}$ soil [‡]	1	94.07 (34.03)a§	31.07 (1.16)b	33.93 (5.43)b	39.67 (6.61)b	0.02
	3	81.03 (2.85)a	29.80 (1.20)b	50.50 (18.50)ab	95.23 (25.71)a	0.01
	5	100.53 (4.07)a	130.63 (9.35)ab	147.43 (20.51)b	159.93 (20.07)b	0.02
	7	169.10 (5.11)a	172.53 (1.65)a	75.23 (31.69)b	54.13 (29.61)b	<0.001
	10	171.93 (5.35)a	152.53 (23.29)ab	124.40 (8.83)b	133.57 (5.02)b	0.03
	13	299.63 (53.61)a	255.73 (11.83)a	259.30 (27.23)a	256.93 (11.06)a	0.47
	16	95.40 (1.70)a	114.90 (3.60)a	119.77 (18.37)a	127.77 (23.28)a	0.24

[†] Control (CTRL); artificial, unlined burrows (ARTF); artificial, litter-lined burrows (LEAF); and *L. terrestris* earthworm burrow (WORM) treatments.

[‡] Initial mean (\pm std. dev.) of total soil bacterial biomass was 39.00 (9.27) $\mu\text{g g}^{-1}$ soil.

[§] Different letters for a particular incubation time indicate a statistically significant difference ($P < 0.05$) among treatments ($n = 3$).

[¶] P values are given for each one-way ANOVA comparing CTRL, ARTF, LEAF, and WORM treatments.

biomass (std. dev.) ranged from 29.80 (± 1.20) to 299.63 (± 53.61) $\mu\text{g g}^{-1}$ soil with values in all treatments converging during the last 6 wk of incubation (Table 2). However, significant differences in total bacterial biomass between CTRL and ARTF occurred during the first 3 wk of incubation and ARTF was similar to the other burrow treatments for the first 10 wk of incubation. Total bacterial biomass in LEAF and WORM was generally significantly different from CTRL for the first 10 wk of incubation, with significantly lower values than CTRL on Weeks 1, 7, and 10. Despite the number of significant differences found in both the active and total bacterial biomass, the active/total bacterial biomass ratios were not significantly different among treatments and showed a general decline with incubation time (data not shown).

Active fungal biomass, which ranged from a mean (\pm std. dev.) of 0.17 (± 0.12) to 7.09 (± 2.82) $\mu\text{g g}^{-1}$ soil, only comprised 1 to 10% of total fungal biomass in all treatments (Fig. 1). Active fungal biomass increased from the start of the incubation in WORM soil and was significantly higher than all other treatments on Week 3 (with mean values of 6.87 [± 4.14] $\mu\text{g g}^{-1}$ WORM soil compared with 0.34 [± 0.07] $\mu\text{g g}^{-1}$ CTRL soil). Mean values for total fungal biomass fell within the range of 30.85 (± 2.85) to 223.90 (± 162.87) $\mu\text{g g}^{-1}$ soil. There were no significant differences in either active fungi or total fungi during the incubation among CTRL, ARTF, and LEAF soils; however, active fungal biomass in LEAF was intermediate between ARTF and WORM values (data not shown). Although total fungal biomass appeared to be greatest in WORM, differences between

WORM and CTRL were significant only on Weeks 3 and 7. Whereas there was a decline in active fungal biomass in CTRL during the last 6 wk of incubation, the ratio of active/total fungal biomass in WORM soil increased, such that the ratio was significantly greater than in all other treatments at the conclusion of the incubation (Fig. 1).

The ratio of active fungal/bacterial biomass ranged from 0.03 to 0.30 in CTRL, 0.09 to 0.69 in ARTF, 0.15 to 0.95 in LEAF and 0.21 to 1.22 in WORM (Table 3). The ratio generally followed the order: CTRL < ARTF < LEAF < WORM.

Nematode Population Dynamics

Total nematode abundance in CTRL and ARTF was low and fairly constant throughout the incubation with values ranging from 1 to 4 g^{-1} soil (Table 4). In contrast, total nematode abundance increased dramatically in LEAF burrows until Week 5 when it was eight times greater (16 g^{-1}) than at the beginning of the incubation. Nematode abundance in LEAF soil was significantly higher than other treatments on Weeks 3, 5, 10, and 16 (Table 4). In WORM soil, total nematode abundance was significantly greater than all other treatments at Week 1, greater than CTRL and ARTF, but lower than LEAF on Weeks 3 and 5, approaching values similar to CTRL soil with further incubation (Table 4).

Closer examination of trophic group composition of nematode populations in LEAF and WORM soil revealed that bacterivores and Tylenchids tended to be the most abundant groups in both treatments (Fig. 2).

Table 3. Active fungal/bacterial biomass ratios for four treatments[†] at different incubation times ($n = 3$).

Incubation time	Treatment				P [‡]
	CTRL	ARTF	LEAF	WORM	
wk					
0	0.21 (0.07)	0.21 (0.07)	0.21 (0.07)	0.21 (0.07)	NA [§]
1	0.04 (0.02)	0.09 (0.01)	0.15 (0.13)	0.23 (0.00)	0.12
3	0.03 (0.01)	0.12 (0.06)	0.30 (0.14)	0.43 (0.25)	0.10
5	0.24 (0.09)	0.69 (0.10)	0.65 (0.33)	0.81 (0.18)	0.09
7	0.30 (0.24)	0.40 (0.14)	0.35 (0.16)	0.37 (0.24)	0.96
10	0.20 (0.16)	0.22 (0.22)	0.35 (0.20)	0.49 (0.10)	0.38
13	0.08 (0.06)	0.27 (0.11)	0.95 (0.70)	1.22 (0.48)	0.08
16	0.04 (0.03)	0.19 (0.15)	0.43 (0.34)	0.65 (0.06)	0.05

[†] Control (CTRL); artificial, unlined burrows (ARTF); artificial, litter-lined burrows (LEAF); and *L. terrestris* earthworm burrow (WORM) treatments.

[‡] P value of one-way ANOVA comparing CTRL, ARTF, LEAF, and WORM treatments for each incubation time.

[§] Not applicable.

Table 4. Mean values (\pm standard deviation) of total nematode abundance (g^{-1} dry soil) in four treatments[†] as a function of incubation time.

Incubation time	Treatment				<i>P</i> [§]
	CTRL	ARTF	LEAF	WORM	
wk					
0	2.33 (0.86)	2.33 (0.86)	2.33 (0.86)	2.33 (0.86)	NA
1	0.35 (0.14)a [‡]	1.43 (0.43)ad	3.14 (0.31)bd	5.71 (1.68)c	0.001
3	0.82 (0.10)a	1.71 (0.59)a	10.63 (1.98)b	6.36 (1.01)c	<0.001
5	1.20 (0.13)a	3.76 (1.32)ac	16.38 (3.45)b	7.38 (0.38)c	<0.001
7	0.52 (0.37)a	3.74 (1.54)a	6.32 (4.81)a	7.19 (6.59)a	0.43
10	1.91 (1.17)a	2.01 (0.61)a	12.64 (6.59)b	1.89 (0.30)a	0.03
13	3.04 (0.39)a	1.14 (0.52)a	6.87 (4.55)a	6.82 (7.14)a	0.48
16	0.84 (0.34)a	1.65 (1.29)a	13.14 (3.28)b	2.46 (1.56)a	<0.001

[†] Control (CTRL); artificial, unlined burrows (ARTF); artificial, litter-lined burrows (LEAF); and *L. terrestris* earthworm burrow (WORM) treatments.

[‡] Different letters for a particular incubation time indicate a statistically significant difference ($P < 0.05$) among treatments ($n = 3$).

[§] *P* value of one-way ANOVA comparing CTRL, ARTF, LEAF, and WORM treatments at each incubation time.

^{||} Not applicable.

There were few omnivore/predaceous nematodes in both LEAF and WORM soil throughout the incubation. Marked differences were apparent in the trophic succession of nematodes between LEAF and WORM soil. Plant parasitic nematodes increased in LEAF soil until Week 5 and then decreased until Week 16, while there was little change in plant parasitic nematode abundance with incubation time in WORM soil (Fig. 2). Bacterivorous nematode abundance increased in LEAF soil from 1 to 5 g^{-1} soil until Week 5, and was highest in LEAF soil throughout the remainder of the incubation. In contrast, bacterivore abundance in WORM soil increased during the first week of incubation, but subsequently declined and remained low. Fungivore numbers increased linearly in LEAF soil, but not until after Week 5 (Fig. 2). In contrast, fungivore numbers remained near zero for the entire incubation in WORM soil. Tylenchid populations exhibited similar trends in LEAF and WORM soil, increasing in abundance until Week 5 and decreasing subsequently. Tylenchids are classified as root hair or fungal feeders (Yeates et al., 1993a). Since no plants were present in the mesocosms, Tylenchids were likely feeding on fungal hyphae, but did not exhibit similar temporal changes in abundance as other fungivores.

Protozoan Population Dynamics

Differences in protozoan abundance among all four treatments were measured on Week 10. Numbers of

protozoa were similar in CTRL and ARTF and significantly lower than in the LEAF and WORM treatments (data not shown). Dynamics of protozoan populations were evaluated in LEAF and WORM treatments over the whole incubation. Although mean abundances were generally higher in WORM soil, there were no statistical differences in flagellate, amoeba, ciliate, or total protozoan abundance between the two treatments for the first 7 wk of incubation (Fig. 3). Further incubation resulted in significantly higher abundance of amoeba in WORM soil after 10 wk and of other groups after 13 wk.

DISCUSSION

Earthworms affect many soil biological, chemical, and physical properties, generally with the end result of stimulating decomposition. One mechanism by which earthworms may affect decomposer communities is by providing a conduit for O_2 through the formation of macropores in burrows (Kretzschmar and Monestiez, 1992; Devliegher and Verstraete, 1997), thereby stimulating aerobic metabolism and growth of microorganisms and microfauna. We hypothesized that if diffusion of O_2 was limiting community structure, then microbial and microfaunal communities in ARTF burrows would be different in size and composition than CTRL soil. However, the presence of macropores in ARTF treat-

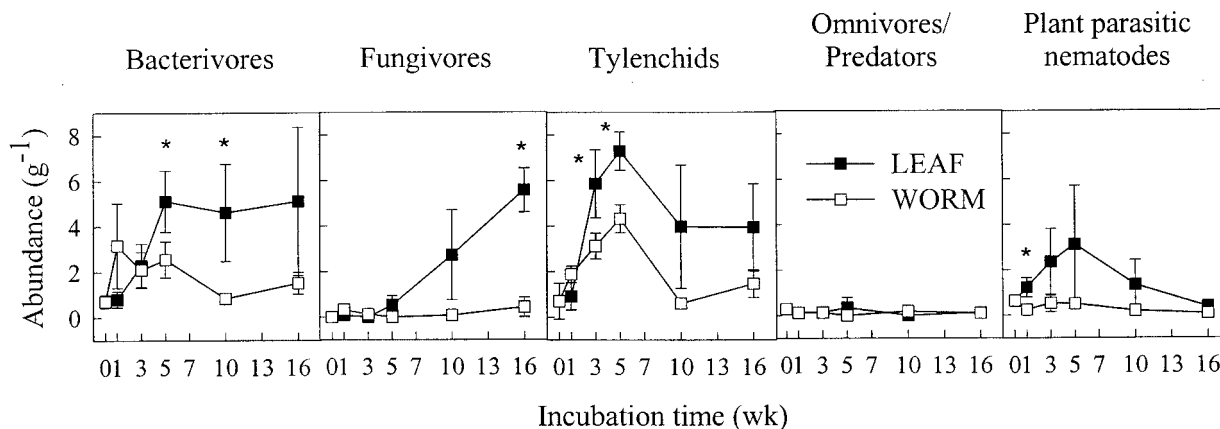


Fig. 2. Bacterivores, fungivores, Tylenchids, plant parasitic and omnivore/predaceous nematodes in artificial burrows lined with corn leaf litter (LEAF) and *L. terrestris* burrows (WORM) treatments as a function of incubation time. Bars represent one standard deviation ($n = 2$ or 3). Asterisks indicate dates that values are significantly higher in LEAF than in WORM soil ($P < 0.05$).

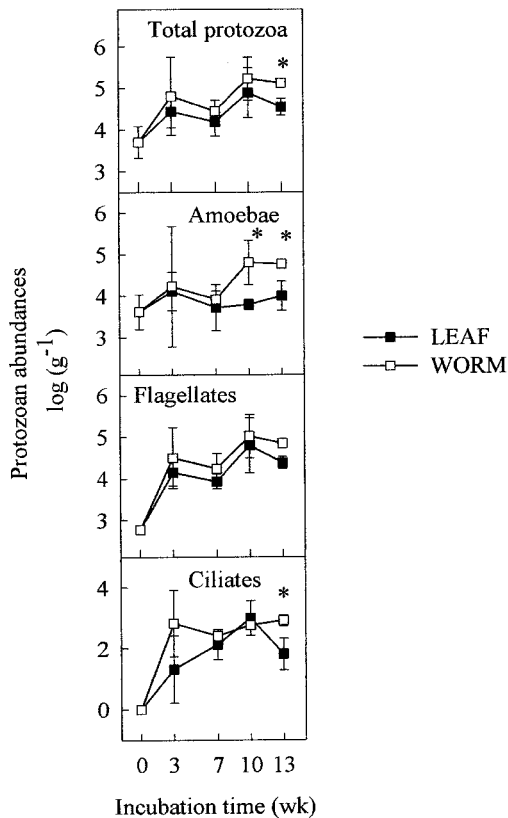


Fig. 3. Log transformed abundance of total protozoa, flagellates, ciliates, and amoebae in artificial burrows lined with corn leaf litter (LEAF); and *L. terrestris* burrows (WORM) treatments as a function of incubation time. Bars represent one standard deviation ($n = 3$). Asterisks indicate dates that values in WORM soil are significantly higher than in LEAF soil ($P < 0.05$).

ments without worms or litter did not alter microbial or microfaunal community structure relative to CTRL soil in any consistent manner. Therefore, diffusion of O_2 did not appear to limit population growth or structure within different levels of the food web in this soil.

In contrast, we observed changes in microbial and microfaunal community composition in the WORM and LEAF treatments, both of which had litter resources available. These results suggested that the incorporation of litter into soil may be one of the more important mechanisms by which earthworms enhance nutrient dynamics. Devliegher and Verstraete (1995) compared microbial numbers and activity in soil with and without litter additions and suggested that increases in microbial populations in soil worked by earthworms were due to nutrient enrichment following litter additions by worm activity. Active microbial biomass and microbivorous grazer abundance would then be expected to be greater in LEAF and WORM burrows than in CTRL or ARTF soil due to the direct contact of the litter with soil microbial decomposers. However, the presence of litter below ground may not be sufficient to explain changes that occur in community structure and microbial function as a result of earthworm activities. If earthworm activities, such as gut transit or physical disruption to the soil structure, have additional impacts on microbial and microfaunal communities in burrows, then populations of

these organisms inhabiting WORM burrow soil should be different than CTRL and LEAF soil.

Grazers of microorganisms have been shown to stimulate an active microbial community (Traunspurger et al., 1997). This is important because much of the microbial biomass in soil may be restricted by lack of resources and generally remain dormant. While increases in microbial biomass in soil worked by earthworms have been measured, microbial activity may be stimulated without a measurable increase in microbial biomass because of simultaneous shifts in populations' size and activity within the total community. There have been reports of increased biomass in earthworm middens (Subler and Kirsch, 1998), but total microbial biomass has also decreased or not changed in earthworm burrows and casts in other studies (e.g., Görres et al., 1997). Therefore, we had hypothesized that, regardless of changes in total microbial biomass, there would be an increase in active microbial biomass, as well as active/total microbial biomass ratios, in the presence of increased microbial biomass. Measurements made on the same mesocosms used in the present study revealed increased C and N mineralization in WORM soil relative to CTRL soil (Amador et al., 2003). These results suggested increased microbial activity, although very few significant differences in total microbial biomass C were observed among treatments. However, there were significant differences among treatments in active and total bacterial biomass. The most notable difference among treatments occurred after a 3-wk incubation, with the highest active bacterial biomass measured in WORM soil (Table 1). Additionally, active fungal biomass was greatest in WORM soil on Week 3. Active/total microbial biomass ratios are significantly higher in WORM soil than CTRL soil on four sampling dates, also supporting this hypothesis (Fig. 1). In contrast, no significant differences among treatments were reflected in the active/total bacterial ratios throughout the incubation. Grazing may lead to differential responses among bacterial populations that are not reflected in calculations derived from coarser-level bacterial biomass measurements. For example, Devliegher and Verstraete (1995) found in one of their studies that proteolytic bacteria increased, while fluorescent pseudomonads decreased in soil affected by earthworms. Shifts in the dominance of microbial functional groups cannot be detected by measuring microbial biomass alone. Molecular analysis of cast and bulk soils has shown decreased diversity and predominance of different species in casts than bulk soil, despite similarities in cast and bulk soil clone libraries (Furlong et al., 2002). These findings may help to explain the contradictory results of studies investigating the effects of earthworms on total microbial biomass measurements (Brown, 1995) and certainly underscore the need to investigate changes in bacterial communities at a greater level of resolution.

Greater abundance of nematodes and protozoa occurred in both LEAF and WORM burrows relative to ARTF and CTRL treatments (e.g., Table 4), supporting the idea that litter in burrows can result in changes in the structure of microfaunal communities. All protozoan

groups were abundant initially and continually in LEAF and WORM soil throughout the 16 wk, albeit with variance in population sizes (Fig. 3). However, despite sustained increases in microfaunal populations in both burrow types containing litter, the dominance of particular trophic groups was dependent on the type of burrow. The presence of earthworms appeared to preferentially sustain enhanced protozoan abundances, especially amoebae, rather than bacterivorous nematode populations. The rapid increase in bacterivorous nematode abundance in WORM soil was not sustained after the first week of incubation (Fig. 2). In contrast, bacterivorous nematodes increased from the start of the experiment until Week 5 and then remained at elevated abundances in LEAF burrows.

Bacterivorous nematodes may not have competed successfully with protozoa in earthworm burrow soil. Protozoa are metabolically more efficient than bacterivorous nematodes (Coleman et al., 1978), can access smaller pore openings, and may have shorter generation times than nematodes (Elliott et al., 1980; Griffiths, 1986). Earthworms can cause shifts in pore structure in burrows toward smaller pore volume and smaller pore openings (West et al., 1991; Görres et al., 2001). In addition, protozoa and earthworms may share a mutually beneficial relationship. Protozoa are considered a food source for earthworms (Miles, 1963; Bonkowski and Schaefer, 1997), but, despite digestion of active protozoa, protozoan cysts benefit from increased distribution and activation in earthworm castings (Bamforth, 1988; Bonkowski and Schaefer, 1997). Our data suggest that, protozoa may have dominated bacterial food web dynamics in WORM burrow soil. In contrast, both bacterivorous nematodes and protozoa were important to bacterial food web dynamics in LEAF burrow soils.

Active fungal biomass was higher in WORM soil than CTRL soil at 3 wk of incubation. In addition, the active/total fungal biomass ratios were significantly higher in WORM soil than other treatments by the conclusion of the incubation (Fig. 1). Earthworms line their burrows with cast material (Lavelle, 1988) and fungi have been observed to grow in casts for up to 15 d following deposition (Parle, 1963a). Moreover, hyphal growth may contribute to increased stability of earthworm cast soil (Parle, 1963a; Haynes and Fraser, 1998). Conversely, despite the fact that fungi are known to be components of earthworm diets (Cooke and Luxton, 1980; Edwards and Fletcher, 1988) and have proliferated in their feces (Shaw and Pawluk, 1986), no changes have been observed in fungal counts in soil excreted by earthworms compared with uningested soil in other studies (Devliegher and Verstraete, 1995; Devliegher and Verstraete, 1997). In this study, stimulation of fungal biomass in WORM burrows occurred, but was not always significant. Increases in active fungal biomass were less obvious in LEAF burrows where the consumption of fungi by higher numbers of fungivores may have confounded differences by removing new growth before it was measured. In contrast to LEAF soil, earthworm activities appeared to negatively affect fungivorous nematodes (Fig. 2).

In both LEAF and WORM treatments, Tylenchid nematode dynamics and changes in active fungal biomass showed similar temporal patterns (data not shown). While Tylenchids are considered root-hair and fungal feeders, in this study they were most likely consuming fungi, since there were no plants present in the mesocosms. In LEAF burrow soil, there was a succession of nematode trophic groups, with numbers of Tylenchida and bacterivorous nematodes peaking at 5 wk of incubation, with Tylenchids subsequently declining, followed by increasing numbers of fungivores from Weeks 5 to 16. Similar to LEAF soil, numbers of Tylenchid nematodes in WORM soil increased early in the incubation. Whether fungal growth on the litter residues stimulated Tylenchids, or Tylenchid grazing of fungi stimulated fungal growth, both groups apparently benefited from the presence of litter in the burrows.

Nematode trophic groups may reflect changes in the pathway of decomposition and availability of food sources (Freckman, 1988; Yeates et al., 1993b). For example, bacterivores preceded fungivores in other studies where fungivores became dominant only after organic matter decomposition slowed and easily degraded organic matter was consumed (Freckman, 1988; Griffiths et al., 1993; Fu et al., 2000). In another study, Yeates (1981) found that the identifications of nematode genera did not change in the presence of earthworms, but changes occurred in the proportions of different nematode trophic groups. For example, both Tylenchids and bacterial feeders decreased while omnivores and root feeders increased in soil influenced by earthworm activity (Yeates, 1981). Earthworms may affect nematode community structure directly by consumption and digestion of nematodes or indirectly by consumption of the microbial food source of microbivorous nematodes (Yeates, 1981). In this study, the succession of nematode trophic groups in LEAF soil may reflect changes in the quality of leaf litter and the success of nematodes with different feeding strategies. In contrast, while the input of leaf litter resources probably stimulated microbes and consequently microfaunal grazers, earthworm activities appeared to counteract increases in microbivorous nematodes in WORM soil (Fig. 2). Fungivorous nematode abundance never increased in WORM soil despite the increase in active fungal biomass, and bacterial feeders decreased to initial levels after 1 wk of incubation.

Additional mechanisms not explored in this study may explain why earthworm effects transcend that of merely adding leaf litter to the soil. Factors contributing to the effects of earthworms on microbial and microfaunal populations may include simultaneously altering biological communities and litter quality during gut passage (Brown, 1995). Worm castings may then serve as nutrient rich deposits in burrows, preferentially benefiting those organisms, which survive gut transport. By this account, nematodes may not be as successful at surviving gut transport as protozoa. The tropical earthworm *Lampito mauritii* exhibited selective feeding preferences on nematode trophic groups and reduced the total nematode abundance in soil pots (Dash et al., 1980). Protozoa, on the other hand, may have a mutually

beneficial relationship with earthworms, supplying enzymatic activity and serving as a food source, with survivors proliferating after excretion (Shaw and Pawluk, 1986). The populations that do survive and proliferate no doubt have tremendous influence on the ensuing dynamics because not only do grazers alter microbial dynamics, but protozoa have different effects depending on both the particular populations present and the types of organic resources available (Rønn et al., 2002). Alternatively, physical alterations of pore structure resulting from burrowing activities may have been more stressful or inhibitory to nematodes than to protozoa. Other processes such as competition may also have driven this dynamic (see review by Brown, 1995).

CONCLUSIONS

Because earthworms alter dynamics on multiple levels of the detrital food web, many biological components must be investigated simultaneously to determine the extent of earthworm effects on soil ecosystems. Our results support the contention that, although the incorporation of litter into burrows significantly increases biological activity, the effect of earthworm activity on soil microbial and microfaunal communities exceeds that of merely incorporating litter into the soil profile. In LEAF burrows, a succession of faunal populations developed through time with increases in protozoa, then bacterivorous nematodes and finally fungivorous nematodes. In contrast, while protozoan abundance was sustained at high levels, bacterivorous nematodes were abundant only initially, and nematode fungal grazers were inhibited throughout the incubation in WORM soil. Further clarification of these population dynamics could lead to significant developments in understanding how ecological interactions affect nutrient availability. For example, we have observed higher nitrate values in WORM than LEAF burrows and data suggested that, although similar mechanisms may be affecting C and N dynamics in artificial and earthworm burrows, additional factors contributed to biogeochemical processes in earthworm burrows (Amador et al., 2003). These additional factors are likely related to ecological interactions promoted and/or inhibited by earthworm activities beyond the effect of litter introduction into burrows.

ACKNOWLEDGMENTS

We thank the URI Facilities and Maintenance crew and Cheryl Tefft for their assistance with the initial soil collection. Elizabeth Downing and Casey Farm in Saunterstown, RI kindly supplied the corn leaf litter. We would also like to acknowledge the work of Heather Taylor, Erika Nicosia and Wilfrid Rodriguez, who were instrumental in collecting laboratory data. This study was funded by a grant from the USDA National Research Initiative Competitive Grants Program, funds from the Rhode Island Agricultural Experiment Station and the University of Rhode Island's Partnership for the Coastal Environment.

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