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Soil invertebrate and microbial communities, and decomposition as indicators of polycyclic aromatic hydrocarbon contamination

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Abstract

Soil organisms are useful for quantification of ecological impact of chemical contamination of soils. This study examined the effects of creosote (complex mixture of polycyclic aromatic hydrocarbons) on composition and abundance of soil invertebrates (nematodes, collembolans and mites) and decomposition processes. Thirty intact soil cores and adjacent litter samples were collected each of three times during the 1998 growing season from soil contaminated with creosote for 50 years. Each core was divided evenly into two subsamples. Abundance of nematodes (by family), Collembola (by family), mites (by Oribatida and others), total bacterial biomass, and active fungal biomass were quantified in the first subsample; soil properties including polycyclic aromatic hydrocarbon (PAH) concentration, organic carbon, pH, electrical conductivity (EC), bulk density, soil moisture and soil texture were measured in the second subsample. Creosote affected soil food webs and decomposition more by altering habitat of microinvertebrates and their prey, fungi and bacteria, than by direct toxicity. We hypothesize that nematodes were affected directly by PAH, more than collembolans or mites, because of their intimate contact with contaminated soil particles and permeable cuticles. Collembola and mites explained decomposition of 100% cellulose and mixed cellulose/lignin substrates better than nematodes because of their co-location in the litter layer. This is the first study to examine effects of PAH contamination on soil food webs and ecological processes.

Keywords: Acari; Collembola; Creosote; Environmental monitoring; Mites; Nematodes; PAH; Springtails

1. Introduction

Soil organisms may be useful in identifying clean-up priorities and monitoring environmental change because they provide objective metrics that integrate physical, chemical, and biological parameters. Thus, they provide a more accurate and feasible measure of biotoxicity of chemical contaminants, such as creosote, than measuring comprehensive profiles of individual chemical concentrations and their degradation products (Debus and Hund, 1997). Creosote is a mixture of thousands of chemicals with 85% as two–five-ring polycyclic aromatic hydrocarbons (PAHs). Compounds with four or more aromatic rings are recalcitrant, and known to be carcinogenic and mutagenic (Mueller et al., 1989). Creosote contamination is a threat to ecosystem health or condition because of its widespread use in railroad ties, electrical and telephone poles, coke production, petroleum refining and other high-temperature industrial processes. In addition, ground reservoirs and tanks of creosote lay abandoned with creosote seeping into surrounding soil and groundwater (Conrad et al., 1999).

Among microinvertebrates, nematodes (Bongers, 1990), Collembola (van Straalen and van Gestel, 1993; Frampton, 1997), and mites (Ruf, 1998) are

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popular candidates as bioindicators because of their role in essential ecological processes of soil including nutrient cycling and decomposition (Wasilewska et al., 1975; Ingham et al., 1985; Beare et al., 1992). The relative abundance and ubiquitous nature of soil invertebrates contribute to their usefulness in comparing their community structure among ecosystem types and land management practices (Neher, 1999a,b, 2001). Of these three groups of microinvertebrates, nematodes have been evaluated for their use as bioindicators most often because of relatively successful sampling methodologies, feeding preference classification, analysis, and interpretation (Yeates et al., 1993; Elliott, 1994; Gupta and Yeates, 1997; Neher et al., 1998). An ecological succession maturity index is available as an ecosystem metric to determine effects of environmental stresses on nematode communities (Bongers, 1990). Since the original maturity index was proposed, various modifications have been suggested (Popovici, 1992; Yeates, 1994; Bongers et al., 1995).

Although evaluated less frequently, mites and collembolans also have potential as bioindicators of soil health or condition. Collembola have been useful for monitoring the process of site rehabilitation by examining their rates of recolonization in soil (Hutson, 1980a,b; Greenslade and Majer, 1993). Because of their large population sizes (10^4 to $10^5\,m^{-2}$), rapid reproduction rates, role in fragmenting detritus, and grazing upon microbes, Collembola have a significant impact on microbial dynamics (Teuben and Roelofsma, 1990; Hopkin, 1997). In addition, mites occupy many trophic levels with varied feeding, reproduction, and dispersal strategies (Dindal, 1990; Coleman and Crossley, 1996; Walter and Proctor, 1999). Mites have been evaluated as potential bioindicators of soils contaminated by wood preservatives, such as pentachlorophenol (PCP) (Salminen and Haimi, 1996). Ruf (1998) developed a maturity index for Gamasina mites in forest soils polluted with heavy metals.

For interpretation of ecosystem health, it is useful to correlate bioindicators to ecological processes critical to ecosystem function. In using organisms, there first needs to be a solid knowledge of the organisms' response to different types of pollution, different combinations of chemicals, and answers to whether or not the organisms are healthy, and not merely surviving (Cairns and Niederlehner, 1993). An ecological process, such as decomposition, may provide an additional assessment of function because it amalgamates soil food web dynamics and environmental variables (Parmelee, 1995), both of which respond to toxic effects of soil pollutants.

The goal of this study was to test four hypotheses: (1) family richness and abundances of soil nematodes, Collembola, and mites will be greater in areas of less rather than more concentrated PAH; (2) nematode communities will shift back to an earlier state of ecological succession, thus reducing maturity index values in contaminated sites; (3) given the fungicidal properties of PAH, decomposition rates of both cellulose and lignin would be altered; and (4) decomposition rates will reflect changes in invertebrate community composition and, thus, serve as indicators of soil health. Previous studies demonstrate effects of PAHs on microbial populations, collembolans, earthworms, Diptera larvae or nematode communities individually (Cutright and Lee, 1994a,b; Snow-Ashbrook and Erstfeld, 1998; Erstfeld and Snow-Ashbrook, 1999; Frouz, 1999; Saterbak et al., 1999; Krauss et al., 2000). This study is novel by examining both microbial and invertebrate communities (nematodes and microarthropods) in combination with decomposition in soils contaminated by long-term exposures to a wide range (5–37,500 ppm) of PAH concentrations.

2. Methods and materials

The 0.5-ha study site was located in the city of Toledo (41°38′00″N latitude, 83°37′05″W longitude) (Blakely, 1999). Soils at the study site had pH 7.2 (±0.38) (mean ± S.D.) and EC of 8.47 (± 2.6) $dS\,m^{-2}$. A creosote reservoir was established at the site about 50 years ago and since then, has been leaking into adjacent soil and groundwater. The site, located about 75 m from the reservoir, consisted of piles of soil contaminated with creosote previously adjacent to the original reservoir that were removed for construction of a warehouse. These soils are vegetated currently by deciduous forest (*Acer negundo* L., *Fraxinus pennsylvanica* Marshall, *Gleditsia triacanthos* L., *Morus alba* L., *Populus deltoides*, *Prunus serotina*, *Quercus coccinea*, *Q. palustris*, *Robinia pseudoacacia*, and *Ulmus americana* L.) with a dense understory of perennial and annual herbs and vines.

Based on the forested vegetation, we estimate the piles were established about 10–20 years earlier.

Samples were taken near the base of trees and shrubs away from any trails or paths to avoid effects of vehicular compaction on bulk density. Thirty intact soil cores (5.1 cm diameter, 7.6 cm depth) and adjacent litter were taken each of three times in 1998 (8 May, 26 June, and 17 August). Each core was divided evenly into two subsamples. Soil invertebrate and microbial communities were characterized in the first subsample. We assumed that bacterial and fungal communities inhabiting the decomposition substrate baits would be similar to those in soil adjacent to litter baskets. Soil cores were stored at existing field moisture levels and 14 °C to minimize changes in soil invertebrate populations prior to assay (Barker et al., 1969). The second subsample was divided for measurement of PAH concentration and soil properties thought to influence soil invertebrate communities, decomposition, and fate of PAHs. Properties measured were organic carbon content (Storer, 1984) with an ashing temperature reduced to 360 °C to avoid dehydroxylation of kaolinitic clays (D.A. Storer, personal communication), pH, EC (Smith and Doran, 1996), bulk density (Sarrantonio et al., 1996), and soil texture (Hillel, 1982). Microarthropods, PAH concentration, gravimetric moisture, and organic carbon content were also quantified in litter.

PAH concentrations were quantified using a Hewlett-Packard 6890 Series II Plus gas chromatograph with 5972 series mass selective detector (Blakely, 1999) and 5M5 5% phenyl methyl siloxane column (30 m × 250 mm × 0.25 mm i.d.). An amount of 3 g of soil or litter were placed in cellulose extraction thimbles in a Soxtec System HT 1043 Extraction Unit Tecator, boiled in methylene chloride for 5 min, refluxed for 1 h, and the extract placed in 2 ml amber vials. Analysis was limited to phenanthrene, fluoranthene, pyrene, and benzo[a]pyrene because they represented the greatest concentrations in preliminary samples of creosote-contaminated soil. An unidentified peak was present on the chromatogram, with a similar retention time and base peak to benzo[a]pyrene. Because the exact nature of this compound is unknown, it is referred to as the "unidentified five-ring PAH" throughout this article, and is included because of its large quantity. Procedures for extracting PAHs from litter were the same as those followed to analyze the soil with one exception. The final litter extract was filtered through a cellulose acetate membrane (0.45 μm, Corning Glass Works, Corning, NY) to reduce the amount of plant litter material that was extracted in the reflux process. Sites were classified into four groups based on total soil PAH concentration measured at each site: (1) 5–100 ppm; (2) 200–800 ppm; (3) 1600–5300 ppm; (4) 11,500–37,500 ppm. Litter PAH concentration categories were assigned as follows: (1) 1–50 ppm; (2) 50–100 ppm; (3) 100–500 ppm; (4) 500–1000 ppm; (5) 1000–3300 ppm; and (6) 39,000 ppm (Blakely, 1999).

Nematodes were extracted from soil by a modified Cobb's sieving and gravity method followed by sugar centrifugal flotation (sensu Neher et al., 1995). Nematodes were enumerated and identified to taxonomic family according to Bongers (1987), Hunt (1993), Nickle (1991), Goodey (1963), Maggenti (1981, 1983, 1991), Maggenti et al. (1987), and Andrássy (1968, 1979, 1980, 1984). Taxonomic families were assigned a colonizer–persister (CP) scale value according to Bongers (1990), in which scores range from 1 (least sensitive, *r*-strategist) to 5 (most sensitive, *K*-strategist), and a trophic group (bacterivore, fungivore, plant-parasite, omnivore, and predator) according to Yeates et al. (1993). Abundance was standardized by gravimetric soil moisture. Two indices were computed: combined plant-parasites and free-living nematode maturity index for CP1–CP5 (ΣMI) (Yeates, 1994) and a maturity index excluding nematode families with CP1 (ΣMI25) (Neher and Campbell, 1996).

Microarthropods were extracted from soil using heptane flotation (Geurs et al., 1991) and from litter using Tullgren funnels (Crossley and Blair, 1991). Heptane extraction was chosen for its relatively high efficiency of extraction and yield of both motile and nonmotile organisms. A preliminary experiment estimated an extraction efficiency of 75% for Collembola, 79% for Oribatida mites, and 64% for non-oribatid mites using heptane flotation; 100% efficiency was defined as numbers in three successive extractions of the same sample. The Tullgren method was chosen for litter to estimate abundances of microarthropods involved actively in decomposition, while avoiding entrapment of excess plant litter in a heptane/water interface. Of the microarthropods extracted, we

enumerated numbers of collembolans by family, mites as Oribatida and non-Oribatida mites, and other microarthropods by order. Subgroups chosen broadly represent the fungivorous community (Siepel and de ruiter-Dijkman, 1993).

Bacterial and fungal biomass was quantified by direct microscopy (Babiuk and Paul, 1970) for all three sampling times with the exception that fungal biomass is not reported for the first sampling period because creosote interfered with an initial high performance liquid chromatography (HPLC) procedure. Active bacterial biomass was measured according to Lodge and Ingham (1991) and active fungal biomass was determined by measuring the length and diameter of FDA-stained hyphae (Ingham and Klein, 1984; Lodge and Ingham, 1991).

Decomposition was quantified using litter baskets (Blair et al., 1991). One basket was placed at each of 30 sampling sites. Two known organic substrates [2.0 cm diameter disks of museum board (100% cellulose) and balsa wood (76% cellulose, 22% lignin)] were secured in fiberglass window screen pouches (1-mm mesh) and placed on the surface of the soil profile and secured under the lid of each litter basket (1-cm mesh) as bait. Ten individual baits were placed in each basket, five of each type. A pair of bait types was harvested every 3 weeks throughout the spring and summer of 1998, and decomposition was estimated as percent mass loss through time.

Spearman correlations and stepwise linear regressions were performed to quantify associations among abundance of soil invertebrates, microbial biomass, concentrations of PAH (phenanthrene, fluoranthene, pyrene, and benzo[a]pyrene), soil properties (organic carbon content, pH, EC, bulk density) and decomposition of cellulose and mixed lignin/cellulose substrates. Data included 30 locations at each of three sampling times ($n = 90$). Taxa present in more than 20% of the samples were included in correlation analyses. Log and arcsine transformations were used to normalize abundance and proportional data, respectively, prior to analysis. Correlation and regression procedures were performed using Statistical Analysis System Software, Version 6.08 (SAS Institute Inc., 1989).

Canonical correspondence analysis (CCA) was performed to explore associations among nematode and microarthropod abundances relative to PAH concentration, soil and litter chemistry (EC, organic carbon, sand content, clay content, pH, bulk density, gravimetric moisture), microbial biomass (active fungi, total bacteria), and decomposition rates (cellulose, mixed lignin/cellulose). A direct gradient procedure was performed with Canoco, Version 4 Software (ter Braak and Smilauer, 1998). Organism abundances were transformed as log $(x + 1)$ prior to analysis. Unrestricted Monte Carlo permutation tests were performed under a reduced model. Separate analyses were run for categories of organisms found in at least 20% of samples: nematodes in soil, microarthropods in soil, and microarthropods in litter. CCA results are displayed graphically with bi-plot scaling focused on inter-species distances, where vectors depict environmental variables and taxa are represented as points. The result is a bi-plot that approximates the weighted averages of each taxon with respect to each of the environmental variables (creosote constituents, decomposition and soil properties). Long vectors correlate more strongly with ordination axes than short vectors, and have greater reliability in predictive application. Ordination axes are presented in sequence of variance explained by a linear combination of environmental variables.

3. Results

3.1. Soil community

Nematodes were more abundant in soil than microarthropods (Table 1). Within nematode communities, Aphelenchoididae, Cephalobidae, Criconematidae, dauer larvae (Rhabditidae), Plectidae, and Rhabditidae were most abundant while Monhysteridae, Hemicyclophoridae, Nordiidae, and Tylencholaimidae were least abundant. Generally, numbers of bacterivores and plant-parasites were greater than numbers of predators and omnivores. Among bacterivores, Cephalobidae and dauer larvae (Rhabditidae) were most numerous. Criconematidae and Hoplolaimidae were the most abundant plant-parasitic nematodes. Finally, predators and omnivores were dominated by numbers of Aporcelaimidae, Mononchidae and Qudsianematidae. Nematode predators, Mononchidae and Aporcelaimidae, correlated negatively with dauer larvae of Rhabditidae ($r = -0.20$, $P = 0.0580$) and

Table 1
Abundance of microinvertebrates per gram of dry soil

Taxon	CP value[a]	Trophic group[b]	Abundance
Total Nematodes			18 (17)
Alaimidae	4	B	0.060 (0.19)
Anguinidae	2	F	0.52 (1.2)
Aphelenchidae	2	F	0.46 (0.95)
Aphelenchoididae	2	F	0.94 (2.4)
Aporcelaimidae	5	O	0.32 (0.95)
Belondiridae	5	O	0.030 (0.13)
Bunonematidae	1	B	0.030 (0.10)
Cephalobidae	2	B	4.1 (4.9)
Criconematidae	3	Pl	2.2 (7.0)
Dauer larvae	1	B	3.2 (12)
Diphtherophoridae	3	F	0.080 (0.22)
Dolichodoridae	3	Pl	0.040 (0.11)
Dorylaimidae	4	O	0.020 (0.18)
Hemicyclophoridae	3	Pl	0.010 (0.040)
Hoplolaimidae	3	Pl	0.77 (4.0)
Leptonchidae	4	O	0.040 (0.12)
Meloidogynidae	2	Pl	0.41 (3.2)
Monhysteridae	1	B	3.0×10^{-3} (3.0×10^{-2})
Mononchidae	4	R	0.27 (0.49)
Nordiidae	4	F	0.010 (0.050)
Nygolaimidae	5	R	0.030 (0.15)
Panagrolaimidae	1	B	0.060 (0.17)
Paratylenchidae	2	Pl	0.24 (0.88)
Plectidae	2	B	0.98 (1.0)
Prismatolaimidae	3	B	0.51 (0.94)
Qudsianematidae	4	O	0.37 (0.55)
Rhabditidae	1	B	1.3 (2.1)
Thornematidae	5	R	0.010 (0.060)
Tripylidae	3	R	0.11 (0.63)
Tylenchidae	2	Pl	0.70 (1.3)
Tylencholaimidae	4	F	0.010 (0.10)
Total mites	–	–	0.33 (0.40)
Oribatida	–	–	0.13 (0.16)
Non-Oribatida mites	–	–	0.20 (0.28)
Total Collembola	–	–	0.13 (0.21)
Isotomidae	–	–	0.03 (0.06)
Sminthuridae	–	–	4.0×10^{-3} (0.01)
Entomobryidae	–	–	0.16 (0.68)
Onychiuridae		–	0.07 (0.17)
Hypogasturidae	–	–	9.0×10^{-4} (0.01)
Tomoceridae	–	–	5.0×10^{-4} (0.010)
Neanuridae	–	–	4.0×10^{-4} (2.0×10^{-3})
Thysanoptera	–	–	5.0×10^{-4} (5.0×10^{-3})
Diptera (A[c])	–	–	1.0×10^{-3} (0.010)
Diptera (L[c])	–	–	0.02 (0.04)
Diplura	–	–	3.0×10^{-3} (0.010)
Symphyla	–	–	0.29 (0.73)
Protura		–	0.03 (0.05)
Coleoptera (A)	–	–	5.0×10^{-4} (3.0×10^{-3})
Coleoptera (L)	–	–	2.0×10^{-3} (0.010)
Homoptera	–	–	0.090 (0.38)

Table 1 (*Continued*)

Taxon	CP value[a]	Trophic group[b]	Abundance
Hymenoptera (A)	–	–	0.030 (0.15)
Hymenoptera (L)	–	–	0.020 (0.17)
Isoptera	–	–	1.0×10^{-4} (1.0×10^{-3})
Chilopoda	–	–	1.0×10^{-3} (4.0×10^{-3})
Pauropoda	–	–	0.010 (0.020)
Diplopoda	–	–	0.010 (0.030)
Total microarthropods	–	–	0.22 (0.51)

Values represent means (±S.D.) of 30 sampling sites and 3 sampling dates combined ($n = 90$).
[a] Bongers (1990) CP scale, in which scores range from 1 (least sensitive) to 5 (most sensitive).
[b] B: bacterivorous; F: fungivorous; Pl: plant-parasitic; O: omnivorous; R: predacious (Yeates et al., 1993).
[c] A: adult; L: larvae.

($r = -0.30$, $P = 0.0048$), respectively, but not other bacterivorous nematodes.

Within microarthropod communities, Isotomidae, Entomobryidae, Symphyla, and total mites were often abundant. Hypogasturidae, Tomoceridae, Neanuridae, Thysanoptera, Isoptera, and Coleoptera adults were the least abundant microarthropods. Total numbers of microarthropods ($r = 0.47$, $P = 0.0028$), Collembola ($r = 0.45$, $P = 0.0045$), and mites ($r = 0.48$, $P = 0.0021$) associated positively with bacterial biomass.

3.2. Litter community

Litter invertebrate community composition was qualitatively and quantitatively different than that of the soil community. For example, Isotomidae and total number of mites were the most abundant microarthropods in litter with Oribatida being more abundant than non-Oribatida mites (Table 2). Coleoptera larvae, Chilopoda, Hymenoptera larvae, and Pauropoda occurred in low abundances.

3.3. Microbial biomass

Fungal biomass associated negatively with concentration of pyrene and fluoranthene, ranging from 2.04×10^{-5} to 6.46×10^{-4} μg biomass per gram of dry soil. Contrary to our prediction, PAH contamination affected bacterial populations oppositely. Bacterial populations correlated positively with increased concentrations of PAH reaching 10^8 to 10^9 μg biomass per gram dry soil.

3.4. Direct effect of PAH contamination on soil communities and decomposition

Even though ΣMI or ΣMI25 values were similar among categories of combined PAH concentration (Table 3), individual nematode families responded to

Table 2
Mean (± S.D.) abundance of microarthropods in litter

Taxon	Abundance
Oribatida	24 (110)
Non-Oribatida mites	11 (23)
Total mites	35 (110)
Isotomidae	10 (45)
Sminthuridae	1.4 (11)
Entomobryidae	0.020 (0.090)
Onychiuridae	0.32 (2.3)
Hypogastruridae	0.10 (0.69)
Tomoceridae	0.060 (0.54)
Total Collembola	2.5 (12)
Thysanoptera	0.12 (0.67)
Larvae Diptera	0.50 (3.4)
Larvae Coleoptera	3×10^{-3} (0.030)
Homoptera	0.25 (1.2)
Adult Hymenoptera	0.11 (1.0)
Larvae Hymenoptera	1.0×10^{-3} (0.010)
Chilopoda	5.0×10^{-3} (0.050)
Pauropoda	2.0×10^{-3} (0.030)
Diplopoda	0.040 (0.34)
Araneida	0.12 (1.17)
Dermaptera	0.050 (0.48)
Total insects	1.2 (3.9)

Abundance values (mean per gram of dry litter) represent means of 30 sampling sites and 3 sampling dates combined ($n = 90$).

Table 3
Maturity index values for individual sites and for the categories of total PAH concentration (mean ± S.D.) in May 1998

Creosote category	*n*	Concentration (ppm)	ΣMI	ΣMI2–ΣMI5
1	11	5–100	2.1 (0.39)	2.3 (0.27)
2	9	200–800	1.7 (0.33)	2.0 (0.25)
3	7	1600–5300	2.3 (0.33)	2.4 (0.29)
4	3	11500–37500	1.9 (0.07)	2.1 (0.22)

PAH contamination. Three types of responses were observed: (1) numbers of *r*-strategists increased and numbers of *K*-strategists decreased; (2) no change in numbers of *r*-strategists but numbers of *K*-strategists decreased; and (3) neither extreme *r*- or *K*-strategists were affected; but instead numbers of an intermediate ecological successional group declined (Table 4). Phenanthrene contamination gave the first pattern. Specifically, proportions of *r*-strategists, CP1, and *K*-strategists, CP4 and CP5, associated positively and negatively with concentrations of phenanthrene, respectively. Not surprising, ΣMI but not ΣMI25 associated negatively with concentration of phenanthrene (Table 4). The second pattern was observed for concentrations of fluoranthene and pyrene. For example, numbers of CP5 nematodes including Aporcelaimidae associated negatively with fluoranthene and pyrene concentration but there was no change in abundance of *r*-strategists. The third pattern was observed for five-ring PAHs, benzo[a]pyrene and the unidentified compound. Specifically, numbers of nematodes with CP values of 2 and 3 (Aphelenchoididae, Paratylenchidae, Criconematidae, Tylenchidae, and Hoplolaimidae) decreased with increasing contamination, while *r*- and *K*-strategists were unaffected (Table 4). Oribatid mites were correlated negatively with smaller-ring PAH (phenanthrene, fluoranthene and pyrene) but not affected significantly by five-ring PAH compounds, benzo[a]pyrene or the unidentified five-ring compound (Table 4). Organisms not mentioned had no

Table 4
Spearman linear correlations coefficients of soil microinvertebrates, bulk density, and PAHs[a]

Invertebrate groups	Bulk density	EC	Phenanthrene	Fluoranthene	Pyrene	Benzo[a]pyrene	Unidentified five-ring PAH
Nematodes							
CP1 (%)	0.41***	NS	0.30**	NS	NS	NS	NS
Dauer larvae (Rhabditidae)(1)[b]	0.38***	NS	NS	NS	NS	NS	NS
Rhabditidae (1)	0.20*	NS	NS	NS	NS	NS	NS
CP2 (%)	NS	0.36***	NS	NS	NS	NS	NS
Anguinidae (2)	−0.27**	NS	−0.22*	NS	NS	NS	NS
Aphelenchoididae (2)	NS	0.26*	NS	NS	NS	−0.25*	−0.23*
Paratylenchidae (2)	0.21*	NS	NS	NS	NS	−0.26*	−0.25*
CP3 (%)	NS	−0.33**	NS	NS	NS	0.31**	0.31**
Hoplolaimidae (3)	−0.25*	NS	NS	NS	NS	NS	NS
Prismatolaimidae (3)	−0.23*	−0.25*	NS	NS	NS	NS	NS
CP4 (%)	−0.37***	NS	NS	NS	NS	NS	NS
Mononchidae (4)	−0.27**	NS	NS	NS	NS	NS	NS
CP5 (%)	−0.49***	NS	−0.34***	−0.32**	−0.33**	NS	NS
Aporcelaimidae (5)	−0.47***	NS	−0.34***	−0.33**	−0.34***	NS	NS
ΣMI	−0.33**	NS	−0.27**	NS	NS	NS	NS
ΣMI25	NS	−0.27*	NS	NS	NS	0.23*	0.24*
Oribatida	−0.36***	0.41***	−0.28**	−0.22*	−0.22*	NS	NS
Non-Oribatida mites	NS	0.44***	NS	NS	NS	NS	NS
Isotomidae	−0.26**	NS	NS	NS	NS	NS	NS

Data represent 30 sampling sites at each of 3 sampling times ($n = 90$). Taxa not listed had no significant association.
[a] NS: $P > 0.06$.
[b] Numbers appearing after nematode families are CP values (Bongers, 1990).
* $P \leq 0.06$.
** $P \leq 0.01$.
*** $P \leq 0.001$.

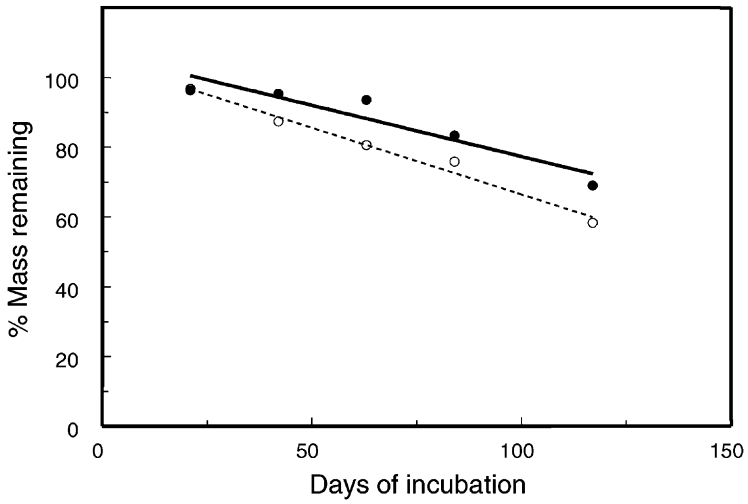

Fig. 1. Mass loss of 100% cellulose (open symbols and dashed line) and mixed cellulose/lignin (solid symbols and line) substrate as a function of days incubation in litterbaskets. Each point represents an average of five baits at each of 30 sampling locations per time ($n = 150$). Lines are determined by linear regression for cellulose ($y = 106.67 - 0.29 \times$ days, $P = 0.0166$, $r^2 = 0.89$) and cellulose/lignin cellulose ($y = 104.7 - 0.38 \times$ days, $P = 0.0011$, $r^2 = 0.98$) separately.

significant correlation with PAH concentration. Decomposition of cellulose was more rapid than mixed lignin/cellulose substrates (Fig. 1) and neither soil nor litter PAH concentration correlated linearly with decomposition of either substrate ($P > 0.05$).

Generally, more groups of microarthropods corresponded with decomposition than nematodes and in opposite ways. Microarthropods (mites, Collembola, Hymenoptera adults, Symphyla, Protura and Pauropoda) associated positively with decomposition of cellulose and/or mixed lignin/cellulose substrates (Table 5). In contrast, CP2 nematodes and numbers of Plectidae and Prismatolaimidae associated negatively with decomposition. No associations ($P > 0.1$) appeared between abundances of other nematodes and decomposition of either substrate.

3.5. Indirect effect of PAH on soil communities and decomposition

Increased concentrations of two–four-ring PAHs [phenanthrene ($r = 0.62$, $P = 0.0001$), fluoranthene ($r = 0.44$, $P = 0.0001$), pyrene ($r = 0.44$, $P = 0.0001$)] correlated positively with bulk density. Whereas, concentrations of five-ring PAHs, benzo[a]pyrene and the unidentified five-ring compound, correlated negatively with EC. Given parallel associations among bulk density, EC and abundances of microinvertebrates, the effects of PAH contamination is confounded with soil properties. Bulk density correlated positively with proportion of relatively small, *r*-strategist (CP1) nematodes and numbers of nematodes in constituent families including Paratylenchidae Rhabditidae, and dauer larvae (a subgroup of Rhabditidae). Conversely, bulk density correlated negatively with larger, *K*-strategist nematodes including Anguinidae, Hoplolaimidae, Mononchidae, Prismatolaimidae, Aporcelaimidae, number of CP4 nematodes, number of CP5 nematodes, and ΣMI. In addition, bulk density correlated negatively with numbers of Oribatida and Isotomidae in soil. A significant positive relationship occurred between EC and Aphelenchidae, Aphelenchoididae, %CP2, Oribatida and non-Oribatida mites, Onychiuridae, Symphyla, Proturans, Hymenoptera adults, and Pauropoda. A significant negative association occurred between Prismatolaimidae, ΣMI25, and %CP3. Indices and taxa not mentioned were neither associated with bulk density nor EC.

Similarly to indirect effects of PAH compounds on soil microinvertebrates, alterations of some soil properties correlated with decomposition rates. In contrast

Table 5
Spearman linear correlation coefficients[a] between nematodes, collembolans, mites, Insecta, and mass loss through time of both substrates ($n = 90$)[b]

Substrate	Invertebrate group	Measure	Cellulose/lignin decomposition	Cellulose decomposition
Soil	Nematodes	Proportion CP2	0.23*	0.22*
		Plectidae	−0.31**	−0.37***
		Prismatolaimidae	−0.21*	−0.27**
	Mites	Oribatida	0.45***	0.51***
		Non-Oribatida	0.61***	0.72***
	Collembolans	Isotomidae	0.20*	0.24*
		Onychiuridae	0.58***	0.67***
		Total	0.54***	0.70***
	Other microarthropods	Larvae Diptera	NS	0.23*
		Symphyla	0.37***	0.39***
		Protura	0.26*	0.25*
		Homoptera	NS	0.29**
		Adult Hymenoptera	0.22*	NS
		Pauropoda	0.44***	0.43***
		Total Insecta	0.33**	0.49***
Litter	Other microarthropods	Total Insecta	0.20*	NS
	Mites	Oribatida	0.53***	0.63***
		Other	0.71***	0.79***
Soil properties		EC	0.43***	0.47***
		pH	−0.33**	−0.44***
		Gravimetric moisture	NS	0.22*
		Organic carbon	NS	0.22*
		Bulk density	NS	NS

[a] NS: $P > 0.06$.
[b] Microinvertebrates not listed had no correlation ($P > 0.05$) with the decomposition of either substrate.
* $P \leq 0.06$.
** $P \leq 0.01$.
*** $P \leq 0.001$.

to other soil properties, EC associated negatively with PAH concentration. Decomposition of both cellulose and mixed lignin/cellulose substrates were affected positively by EC, negatively by soil pH, and not affected by bulk density (Table 5, Fig. 2). Moisture and organic carbon content correlated positively with decomposition of cellulose but not lignin/cellulose mixtures (Table 5, Figs. 2 and 3). Generally, indirect effects of soil properties on microarthropod communities better explained decomposition rates than concentration of PAHs (Figs. 2–4).

4. Discussion

This is the first study examining effects of creosote contamination on soil food webs and ecological processes. To our surprise, creosote affected soil ecosystems more by altering soil properties than direct toxicity. Retention and bioavailability in the environment are reduced by hydrophobicity and organic carbon contents exceeding 2% (Alexander, 2000; Krauss et al., 2000). PAHs sorb to soil particles tightly thereby increasing the bulk density of soil. Greater bulk density means less oxygen and nutrient transportation through soil, thus fewer indigenous flora are able to reach contaminants to degrade them even under optimum conditions. Sorption is often greater for aged contamination; some compounds are adsorbed so strongly they do not show toxicity even though total concentrations exceed recommended guideline values (Weissenfels et al., 1992). Through aging, organic molecules may gradually move into nanopores or micropores (0.3–1.0 nm),

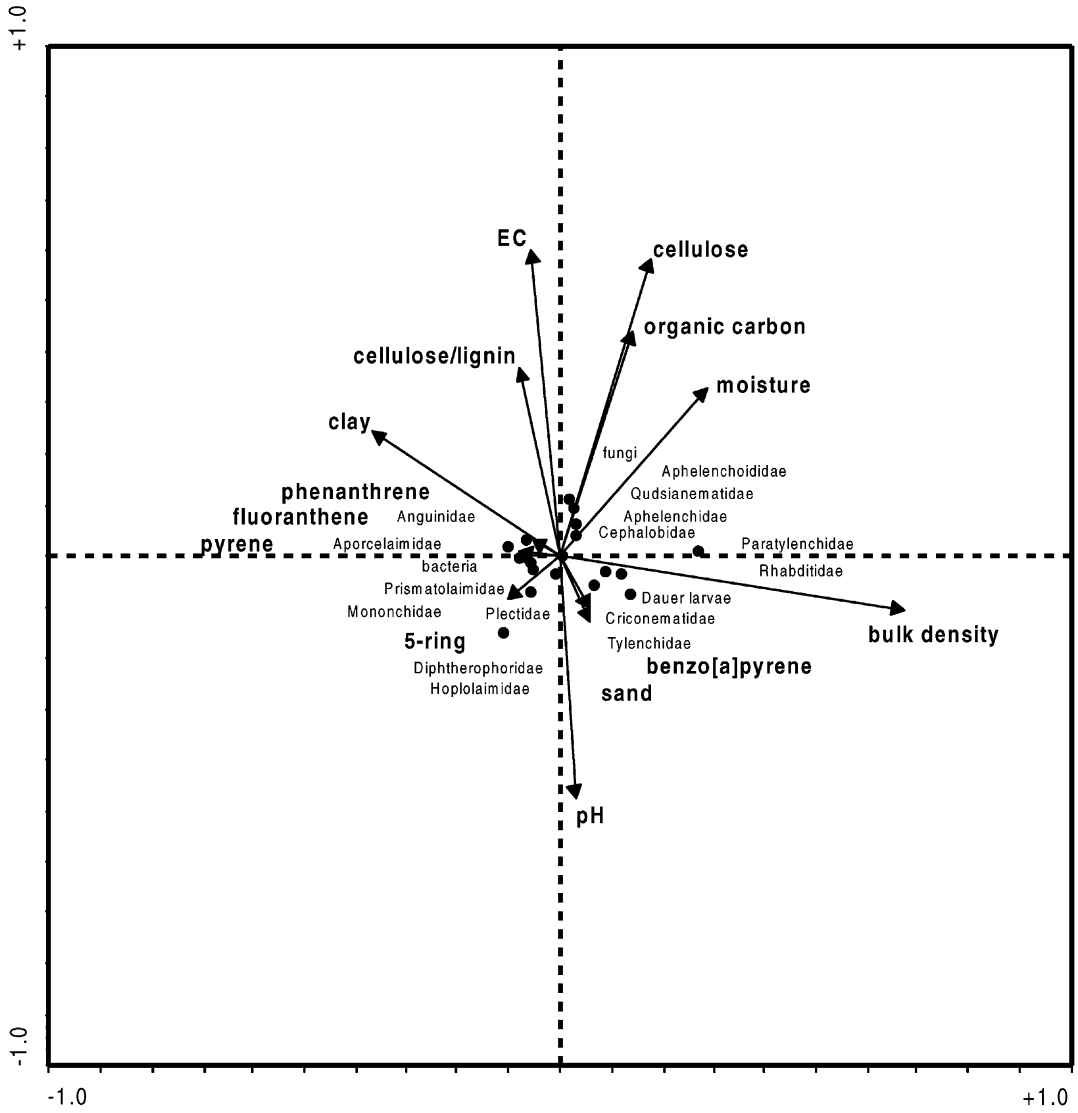

Fig. 2. CCA bi-plot of soil nematode families, soil microbial biomass (active fungi, total bacteria), and environmental variables. Environmental variables including substrate decomposition rate (cellulose, cellulose/lignin), PAH (phenanthrene, pyrene, fluoranthene, benzo[a]pyrene, and five-ring PAH) and soil properties (bulk density, pH, EC, organic carbon content (%), clay content (%), sand content (%), and gravimetric moisture) are illustrated as vectors. Points represent numbers of nematodes; abundances decrease with increasing distance from each point in a unimodal fashion (ter Braak and Smilauer, 1998). Data represent all sites and sampling times combined ($n = 90$). Eigenvalues (lambda) are 0.079 ($P = 0.0750$), 0.046, 0.038, and 0.027 for first (horizontal), second (vertical), third and fourth axes, respectively.

partitioning into soil organic matter, or have strong surface adsorption becoming inaccessible to even bacteria and their extracellular enzymes, thus reducing bioavailablity by limiting diffusion out of these remote sites (Hatzinger and Alexander, 1995; Alexander, 2000). PAHs sorbed to humic substances that are not available for organisms, even though humic substances increase water solubility and mobility of hydrophobic pollutants (Wahle and Kördel, 1997).

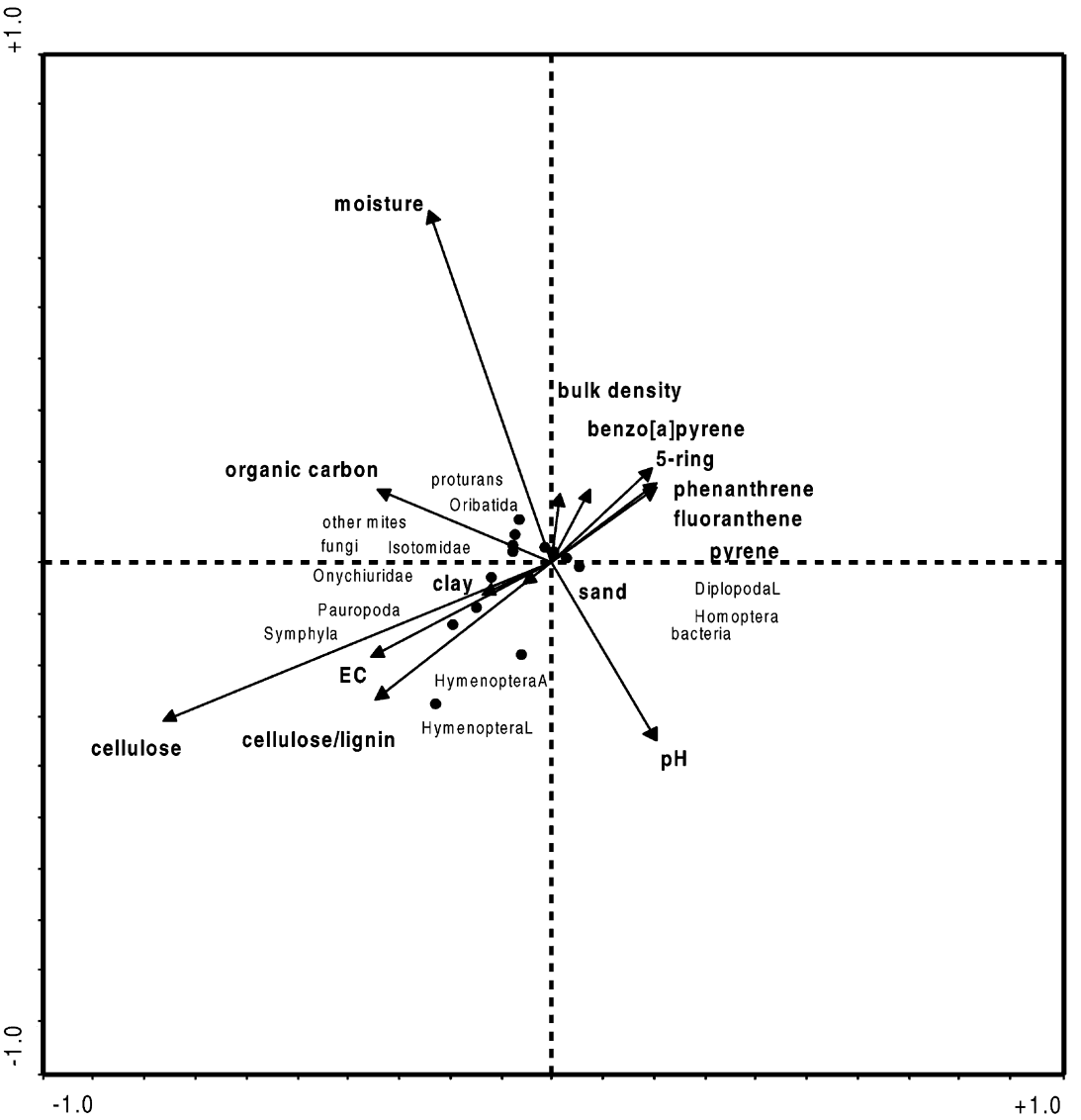

Fig. 3. CCA bi-plot for soil microarthropods, soil microbial biomass (active fungi, total bacteria), and environmental variables. Environmental variables including substrate decomposition rate (cellulose, cellulose/lignin), PAH (phenanthrene, pyrene, fluoranthene, benzo[a]pyrene, and five-ring PAH) and soil properties (bulk density, pH, EC, organic carbon content (%), clay content (%), sand content (%), and gravimetric moisture) are illustrated as vectors. Points represent numbers of collected soil microarthropods; abundances decrease with increasing distance from each point in a unimodal fashion (ter Braak and Smilauer, 1998). L and A at the end of names represent larval or adult stages, respectively. Data represent all sites and sampling times combined ($n = 90$). Eigenvalues (lambda) are 0.118 ($P = 0.1750$), 0.036, 0.023, and 0.010 for first (horizontal), second (vertical), third and fourth axes, respectively.

4.1. Nematoda

The presence of all five trophic groups across the entire range of creosote contamination suggests that creosote contamination is responsible for changes in ecological succession of nematodes communities. Contrary to our predictions, creosote affected ecological maturity indirectly by altering the physical habitat

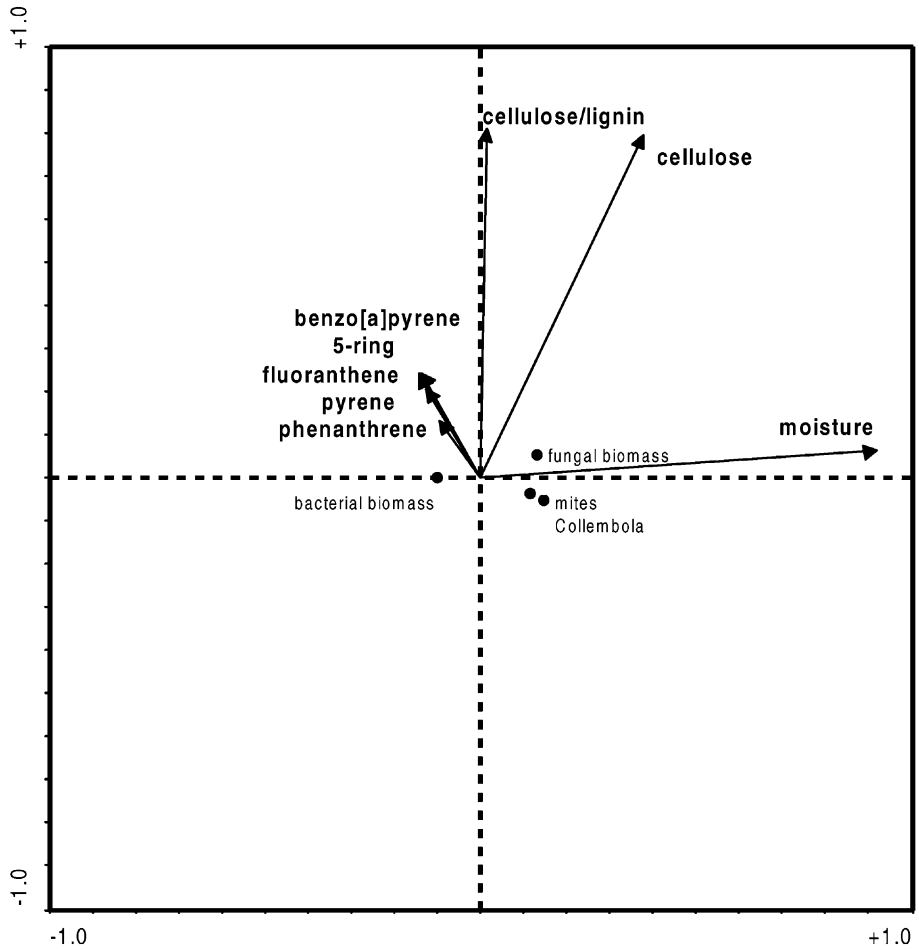

Fig. 4. CCA bi-plot for numbers of total mites and collembolans in litter, soil microbial biomass (active fungi, total bacteria), and environmental variables. Environmental variables including substrate decomposition rate (cellulose, cellulose/lignin), PAH (phenanthrene, pyrene, fluoranthene, benzo[a]pyrene, and five-ring PAH) and gravimetric moisture are illustrated as vectors. Points represent numbers of collected litter microarthropods; abundances decrease with increasing distance from each point in a unimodal fashion (ter Braak and Smilauer, 1998). Data represent all sites and sampling times combined ($n = 90$). Eigenvalues (lambda) are 0.149 ($P = 0.0050$), 0.010, 0.005, and 0.373 for first (horizontal), second (vertical), third and fourth axes, respectively.

of the nematodes rather than the direct chemical toxicity of PAHs. Increased bulk density of soil and hydrophobic properties of PAHs decrease the amount of habitable space within soil pores (Gao et al., 1998) as reflected by decreased ΣMI values, abundance of Paratylenchidae (CP2) with a relatively smaller body size, and depressed number of nematodes assigned CP5 values that have larger body sizes (e.g. Aporcelaimidae). Numbers of bacterivorous nematodes proliferated in soils contaminated with PAHs, while fungivorous nematodes and other trophic groups were reduced. Similar patterns have been observed for soil contaminated by PCP, methyl bromide and heavy metals (Weiss and Larink, 1991; Yeates and van der Meulen, 1996; Salminen and Haimi, 1997). Fungivorous nematodes may have been reduced because their food source was either eliminated or contaminated by the fungicidal properties of creosote. Predatory nematodes were the least abundant trophic group in this study, and may have been present simply because

their prey, bacterivorous nematodes, were prolific (Wardle and Yeates, 1993). However, abundances of omnivores/predators, Mononchida and Enoplida, have been reported previously as abundant in soils contaminated by PAH (Snow-Ashbrook and Erstfeld, 1998). Other *K*-strategists have been reported to be insensitive to cadmium and PCP (Kammenga et al., 1994). Insensitivity to soil contamination can be explained by nematodes exhibiting plasticity in various traits. For example, the nematode *Plectus acuminatus* is able to maintain consistent population growth rates for a range of PCP concentrations. This suggests that toxicants may be regarded as additional environmental factors such as temperature, light, or nutrient availability, which moderate various phenotypic characteristics (Kammenga and Riksen, 1996). At the study site, nematode communities have had 50 years to adapt physiologically or genetically to the contaminants.

It was difficult to separate whether the effect of PAHs on nematode communities was primarily physical or chemical. Paratylenchidae were correlated positively with bulk density, but correlated negatively with the unidentified five-ring PAH and benzo[a]pyrene, which could signify a chemical affect from larger PAH molecules. Popham and Webster (1980) found that cadmium is directly toxic to *Caenorhabditis elegans* (Rhabditidae) by interfering with nutrient uptake and assimilation, not its habitable pore space. Controlled experiments are necessary to determine if PAHs have a direct chemical affect on nematodes or an indirect physical affect through alteration of their habitats.

4.2. Microarthropods

Our findings support previous reports demonstrating reduction in abundance of total Acarina with PAH contamination and Oribatida with metal ores (Erstfeld and Snow-Ashbrook, 1999; Skubała, 1999). Two possible explanations for these negative relationships are that: (1) PAHs are fungicides, thus contaminating and eliminating the microarthropod's fungal or detrital food; and/or (2) the increased bulk density from PAH contamination reduces habitat space for microarthropods. These explanations that focus on either the chemical affect to a nutrient source or alteration of physical habitat may be confounded. For example, abundance of Oribatida was greater in litter than soil, but litter was less contaminated and provided larger pore spaces than soil.

Unlike Greenslade and Majer (1993), we found very few Entomobryidae. In contrast to Erstfeld and Snow-Ashbrook (1999) who noted that the abundance of collembolans increased with PAH contamination, we found no direct association with PAH. Collembola are recognized as primary colonizers and community composition changes through time following disturbances, such as bauxite and coal mining (Greenslade and Majer, 1993). Indirectly, creosote contamination increased bulk density and, thus, indirectly decreased collembolan habitat reducing numbers of Isotomidae, but this trend was not seen for other collembolan families. Metal contamination of soil has also been demonstrated to affect certain families of Collembola more than others (Bruce et al., 1997; Filser and Holscher, 1997). It is difficult to identify mechanisms involved because of the coarse taxonomic resolution used in this study. Finer resolution to the species level would help determine how certain mite and collembolan taxa were affected more than others by creosote contamination (Kohler, 1992). Different species within one family or order may have contrasting responses to PAH stress (e.g. Cephalobidae).

4.3. Decomposition

Measuring decomposition may provide a simple estimate of ecosystem function and is not as tedious or impossible as identifying all the species participating in decomposition. Decomposition reflects changes in microbial and invertebrate populations and communities. Generally, decomposition rate at the study site (cellulose 0.24–0.69% loss/day; lignin/cellulose mixture 0.19–0.62% loss/day) were slower than in other studies measured either with litter baskets (Neher et al., 2002) or litter bags in other temperate deciduous forest soils. For example, cellulose degraded 36.3% and lignin 8.1% within 12 weeks (Entry and Backman, 1995) compared to 31.0 and 41.7% total mass loss of cellulose and lignin/cellulose (initially 22% lignin, 76% cellulose), respectively, during our study of 15 weeks. For future experiments that use decomposition as a measure of soil health, we recommend using more litter baskets than in this study ($n = 30$) and collecting substrate baits at 5-week rather than 3-week intervals. These design modifications account

for bait damage and loss due to the melting of creosote components and improve efficiency of estimating decomposition rates impeded by contaminants such as PAH.

Both bacteria and fungi are capable of degrading small molecular weight PAHs and their relative contributions may be confounded if both are present (Mahmood and Rao, 1993). Bacteria can utilize two–four-ring PAHs as a direct source of energy (Sack et al., 1997) and fungi can decompose two–four-ring compounds in the presence of other carbon sources (Davis et al., 1993; Gramss et al., 1999). Typically, only fungi are capable of degrading five-ring PAHs because of their recalcitrant and hydrophobic nature (Cerniglia, 1993). Both lignin-degrading white-rot and some non-white-rot fungi (*Aspergillus niger*, *Penicillium glabrum*, zygomycete *Cunninghamella elegans*, basidiomycete *Crinipellis stipitaria*) have been shown to degrade a variety of pollutants including PAH, DDT, PCP, and TNT (Haemmerli et al., 1986; Moen and Hammel, 1994; Cutright, 1995; Collins et al., 1996; Wunder et al., 1997; Eggen and Sveum, 1999). Decomposition in PAH contaminated soils are affected by oxygen and nutrient availability, water solubility, number of aromatic chains, and competitive inhibition among PAH compounds (Bouchez et al., 1999). Degradation of PAH with fewer numbers of aromatic rings [e.g. fluoranthene (3), phenanthrene (3), pyrene (4)] is more rapid than those with five aromatic rings (e.g. benzo[a]pyrene) (Cerniglia, 1993). Because of the strong negative association between PAH concentration, fungal biomass, and fungivorous organisms, we do not believe that the fungal community at the study site was a major player in the degradation of PAHs (Canet et al., 2001). Fungi were not eliminated by PAHs, because fungivorous microarthropods were present. Interestingly, prolific bacterial biomass was not associated significantly with decomposition. PAHs provide an abundant carbon source and also increase the surface area of soil particles creating more pockets for bacteria to proliferate and escape predation. This causes a decrease in bacterial mobility by preventing access to plant litter, thereby reducing decomposition rates (Rossell et al., 1973; Bouchez et al., 1996). Entry and Backman (1995) also observed no correlation between active bacterial biomass and degradation of either cellulose or lignin after 12 weeks.

Although bacteria were not correlated with decomposition, bacterivorous nematodes associated negatively with mass loss of both substrates. Alkemade et al. (1993) noted fast decomposition rates with bacterivorous nematodes, particularly the monhysterid species. Bacterial biomass and numbers of bacterivorous nematodes were related inversely suggesting that the peaks of their populations are temporally asynchronous (Neher, 1999a). The hypothesis for asynchronous population peaks is supported by Salminen and Haimi (1997) who show that PCP decreases bacterial biomass, but does not affect bacterivorous nematodes. However, in contrast to Salminen and Haimi (1997), both bacterial biomass and bacterivorous nematodes flourish in soils contaminated with PAHs. Our data support Neher (1999a) rather than Salminen and Haimi (1997).

Ordination analyses suggest that microarthropod communities, especially those in the litter layer, are associated more directly with decomposition than nematodes. This was expected because decomposition was measured at the soil surface where litter microarthropods were collected. Collembola and Oribatida affect rates of litter decomposition by fragmenting the litter to increase surface area for the colonization of microbes (Moore et al., 1987; Dilly and Irmler, 1998). Numbers of Oribatida were related inversely to the decomposition of cellulose perhaps suggesting a trophic cascade effect by grazing on fungi indirectly, thus, decreasing decomposition. Many microarthropods including fungivores (Collembola, total mites, and Pauropoda) and predators (non-Oribatida mites, Diptera, Hymenoptera and Symphyla) correlated positively with decomposition of one or both substrates. For example, non-Oribatida mites and Diptera larvae associated positively with cellulose decomposition; whereas, adult and larvae Hymenoptera in soil associated positively with the decomposition of both cellulose and mixed lignin/cellulose substrates. Furthermore, as predators of microbial grazers, Diptera larvae, Hymenoptera, and Symphyla decrease rates of lignin/cellulose decomposition (Frouz, 1999).

The positive association between EC and decomposition of a mixed lignin/cellulose substrate suggests that EC reacts chemically with PAH to exert an indirect affect on the decomposer organisms. EC measures the presence of ions, which associates negatively with concentrations of polar or neutral PAH molecules.

Thus, increased EC decreases the impact of PAH contamination on organisms sensitive to osmolarity, allowing them to participate in decomposition processes. Another major indirect effect of PAH contamination is by increasing the bulk density of the soil. This partially determines which organisms could be present to participate in decomposition by altering their habitat.

5. Conclusions

We recommend nematodes as a better indicator for PAH contamination of soil than microarthopods because of their intimate contact with the soil particles and contamination, permeable cuticle, and relative knowledge of their taxonomy and trophic groups. In addition, maturity indices of nematode communities have practical and useful application to soils with PAH contamination. Alternatively, microarthropods may serve as a better indicator of decomposition than nematodes. Better judgment on the utility of microarthropods as bioindicators will be possible with finer taxonomic resolution in identification.

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