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Degradation and Mineralization of the Polycyclic Aromatic Hydrocarbons Anthracene and Naphthalene in Intertidal Marine Sediments†

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The degradation of the polynuclear aromatic hydrocarbons (PAHs) anthracene and naphthalene by the microbiota of intertidal sediments was investigated in laboratory studies. No mineralization of either PAH was observed in the absence of oxygen. Both rates and total amounts of PAH mineralization were strongly controlled by oxygen content and temperature of the incubations. Inorganic nitrogen and glucose amendments had minimal effects on PAH mineralization. The rates and total amounts of PAH mineralized were directly related to compound concentration, pre-exposure time, and concentration. Maximum mineralization was observed at the higher concentrations (5 to 100 μg/g [ppm]) of both PAHs. Optimal acclimation to anthracene and naphthalene (through pre-exposures to the compounds) occurred at the highest acclimation concentration (1,000 ppm). However, acclimation to a single concentration (100 ppm) resulted in initial relative mineralization rates over a range of re-exposure concentrations (1 to 1,000 ppm) being nearly identical. Maximum mineralization of both PAHs occurred after intermediate periods (1 to 2 weeks) of pre-exposure. The fraction of the total heterotrophic population capable of utilizing anthracene or naphthalene as sole carbon source was also greatest after 2 weeks.

Several environmental variables have been found to influence organic pollutant degradation in the various terrestrial and aquatic systems examined. These include temperature (26), pH (7, 11), salinity (2, 5, 22, 27), and, in particular, oxygen availability (1, 8). Also found to be important in pollutant degradation are compound-related factors which may select for that fraction of the microbial population which is tolerant of, or adapts to, a compound and degrades it. Such factors include organic pollutant concentration (4, 23), pre-exposure time, and concentration (15, 22, 23, 25).

Relatively little work has been performed on factors controlling the biodegradation of aromatic pollutants in marine environments. This may be due to preliminary observations that the degradation of several pollutant compounds appeared to decrease with increasing salinity (2, 5, 22, 27). However, several studies which have examined the degradation of aromatic pollutants by the indigenous microbiota of marine sediments have found degradation to be comparable to that in other systems (10, 16, 20).

The present study was carried out to examine environmental and compound-related factors which may affect the degradation of two polycyclic aromatic hydrocarbons (PAHs), anthracene and naphthalene, in intertidal marine sediments.

MATERIALS AND METHODS

Sampling and sample preparation. Sediment for all laboratory studies was obtained from an intertidal mudflat of Flax Pond Saltmarsh, Old Field, N.Y. Characteristics of the sediment have been previously described (3).

Sediment for aerobic studies was obtained by scraping the top 0 to 2 mm of sediment with a sterile spatula and placing it in a sterile glass beaker. For anaerobic studies, sediment was obtained in a similar manner from a 5- to 10-cm depth. Flax Pond sea water (salinity, about 28‰) was obtained from Flax Pond Laboratory where it had undergone coarse (10 μm) filtration through nylon cartridge filters. All samples were returned to the Marine Sciences Research Center where they were processed within 1 h.

Sediments were diluted 1:2 (wet wt/vol) with 0.45-μm (Gelman type GA-6) filtered seawater. The resultant slurry was then passed through a 760-μm mesh to remove pieces of shell debris and plant material. Subsamples of slurry were taken and the dry weight of sediment was determined.

In cases where anoxic sediments were utilized, all diluent seawater was purged of oxygen by bubbling with nitrogen gas for at least 30 min before dilution. All vessels containing anoxic slurries were immediately stoppered (butyl rubber; Thomas Scientific, Philadelphia, Pa.) after dispensing, and the headspace was purged with nitrogen gas for 1 min. All experimental slurries were maintained at 25°C in the dark in temperature-controlled water baths with rotary shaking to ensure rapid dispersal of pollutants and equilibration between gas and liquid phases.

Bacterial enumeration. The fraction of the total microbial population capable of utilizing (or tolerating) the two pollutants as sole carbon source was evaluated in a spread-plating experiment. Single-batch enrichments (125 ml) were amended with either 100 ppm (or 100 μg of PAH per g of dry sediment) of the organic pollutant or an equivalent amount of acetone carrier. At time zero (immediately after treatment), 2 weeks, and 4 weeks, subsamples from the enrichments were serially diluted in autoclaved seawater, using sterile techniques. A 0.1-ml portion of 10^2-, 10^3-, and 10^4-fold dilutions was plated onto nutrient agar media and incubated in the dark at 25°C for 3 to 7 days before colony enumeration. Subsamples of each batch enrichment were also taken at each time point for determination of total microbial numbers by acridine orange direct count as modified for

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sediments by Rublee et al. (19). Only those plates containing 30 to 300 colonies were used in data analysis. Sterile undefined medium was prepared by adding the following to filtered (Whatman type GF/F) 90% seawater (on a per-liter basis): 0.3 g of NH_4Cl, 0.5 g of phosphate buffer (containing 5% [wt/vol] each KH_2PO_4 and K_2HPO_4, pH 7.5), 10 ml of trace metal solution, and 1.5% agar (Difco Laboratories, Detroit, Mich.).

Degradation and mineralization of anthracene and naphthalene. Mineralization of anthracene and naphthalene was measured by supplementing 2.5 to 5.0 ml of 1:2-diluted sediment with ^{14}C-labeled PAHs. A total of 1 to 1,000 ppm of the unlabeled compound was added to incubation vials containing sediment slurry. Vials were then sealed with butyl rubber stoppers. Later experiments used stoppers lined with Teflon tape to prevent absorption of organics by stoppers.

For time and concentration dependence of microbial acclimation to PAHs, 10-ml sediment batch enrichment were prepared and dosed as in the plating experiment. At selected intervals, 5-ml subsamples were withdrawn and placed in 20-ml incubation vials, and the appropriate amounts of ^{14}C-labeled PAHs were added {[1(4,5,8)-^{14}C]naphthalene, 5 mCi/mmol, and [9(10)-^{14}C]anthracene, 5 mCi/mmol [Amersham Corp., Arlington Heights, Ill.]}. The use of single large-batch enrichments for acclimating sediment slurries was deemed acceptable on the basis of preliminary analyses of variance of microbial activities in these flasks (data not shown).

Five or 10 μl of the ^{14}C-labeled compound in acetone was added through the stopper via a microliter syringe (final, 0.05 to 0.15 μCi/vial). Preliminary studies showed [^{14}C]naphthalene mineralization to be stimulated by acetone concentrations between 0.4 and 1.0% (vol/vol) and inhibited by acetone concentrations of >1.0% (data not shown). For this reason acetone addition was limited to 0.2% or less in all degradation experiments.

At selected intervals, vial headspaces were flushed with compressed air or nitrogen gas and the $^{14}CO_2$ mineralized from ^{14}C-labeled compounds was quantified. This was performed by placing incurrent and excurrent needles through the stoppers, purging the headspace by passing air or nitrogen through the incubation vials, trapping the effluent gas in 10 ml of a combination $^{14}CO_2$ trapping agent (Oxosol phenethylamine/scintillation cocktail; National Diagnostics, Somerville, N.J.), and counting the samples on a Packard Tri-Carb 300C liquid scintillation counter.

The same $^{14}CO_2$ trapping vial was reused for sequential trapping of the headspace of its respective incubation vial for the duration of an experiment, counting each vial after each trapping. Over the course of an experiment, the capacity of the trapping agent was not exceeded (Bauer and Capone, manuscript in preparation). This procedure saved both time and material. During initial experiments, an additional headspace gas sample was collected in the trapping vial after acidification of the slurry with 0.2 ml of 2 N H_2SO_4 (3). Since no significant increase was noted over the cumulative counts before acidification, this procedure was discontinued.

Rates of mineralization were determined from linear portions of $^{14}CO_2$ production curves. Lag periods and maximum amounts mineralized were also recorded. Duplicate autoclaved controls were run in selected experiments to account for any volatilized ^{14}C-labeled organics in the headspaces. To ensure that headspace oxygen was maintained at the desired levels throughout experiments, headspace subsamples (50 to 100 μl) were analyzed for O_2 on a Shimadzu model GC-R1A gas chromatograph equipped with an electron capture detector. Values for peak height response were compared with values obtained by injecting quantities of known O_2 standards.

The disappearance of parent compounds was followed by incubating identically prepared subsamples with 1 to 1,000 ppm of organic compounds which were not supplemented with ^{14}C-labeled compounds. Incubations were performed in 20-ml vials with Teflon-lined screw caps under the previously described conditions. Because the Teflon did not form a gas-tight seal, anoxic incubation vials were placed in a second glass bottle which was then stoppered and gassed with nitrogen gas. At selected intervals caps were removed, incubations were terminated, and parent compounds were extracted by adding 2.5 to 5.0 ml of acetonitrile (CH_3CN) and replacing the caps. Extractions were performed at room temperature with periodic shaking for 24 to 48 h. At the completion of extraction, samples were placed in 20-ml Corex (Corning Co., Corning, N.Y.) centrifuge tubes and centrifuged at 2,000 rpm for 10 min. The supernatant was then decanted, diluted 100% with distilled water, and eluted through a prepared C_{18} Sep-Pak (Waters Associates, Milton, Mass.) for sample cleanup. Retained compounds were re-eluted in 2.0 ml of methylene chloride (CH_2Cl_2). Subsamples (5 to 100 μl) of this concentrated eluent were injected into a Water's high-pressure liquid chromatograph equipped with an Adsorbosphere (Applied Science, State College, Pa.) C_{18} (5-μm mesh size) column (15-cm length) and detected on a Water's model 440 adsorbance detector (254 nm). Flow conditions were 80% methanol–20% water at 2.0 ml/min. Compounds were quantified by comparing area readout with that of known standards. Standard response was linear over the concentration range used. Recoveries of the compounds were determined by mixing known amounts of organics with sediment slurries and immediately extracting. Recoveries were as follows (mean percentage of total added ± standard error; $n = 2$): anthracene, 89 ± 8%; naphthalene, 76 ± 6%.

Effects of environmental factors. The environmental factors which were examined for their effects on mineralization of ^{14}C-labeled anthracene and naphthalene included temperature, nutrients (nitrate and glucose), and oxygen tension. Temperature was controlled by incubating previously described subsamples at 10, 20, or 30°C in water baths with rotary shaking. Nitrate was added to incubations at final concentrations of 0, 50 μM, 500 μM, or 5 mM in sterile seawater solutions.

Three types of glucose amendments were made. In one series, a single injection (100 μg/g [dry weight] was made to an enrichment flask at time zero of the experiment. At time zero, 1 week, and 2 weeks, triplicate subsamples were taken and redosed with 100 ppm of anthracene or naphthalene plus 10 μl of ^{14}C-labeled PAH stock. Amounts of ^{14}C-labeled compounds mineralized were compared with identical subsamples from an unenriched batch flask. In a second series, daily 100-ppm (5-μl) injections of glucose were made to triplicate 5-ml samples which contained 100 ppm of anthracene or naphthalene plus 10 μl of ^{14}C-labeled organic (controls received daily 5-μl injections of carrier, i.e., autoclaved seawater). In a third series, glucose amendments were identical to the second experiment except subsamples had been acclimated for 1 and 2 weeks with 100 ppm of naphthalene or anthracene, respectively.

The effects of oxygen availability on ^{14}C-labeled anthracene or naphthalene mineralization and parent transformation were assessed in three experiments. The first of these exposed duplicate 5-ml 1:2-diluted sediment slurries

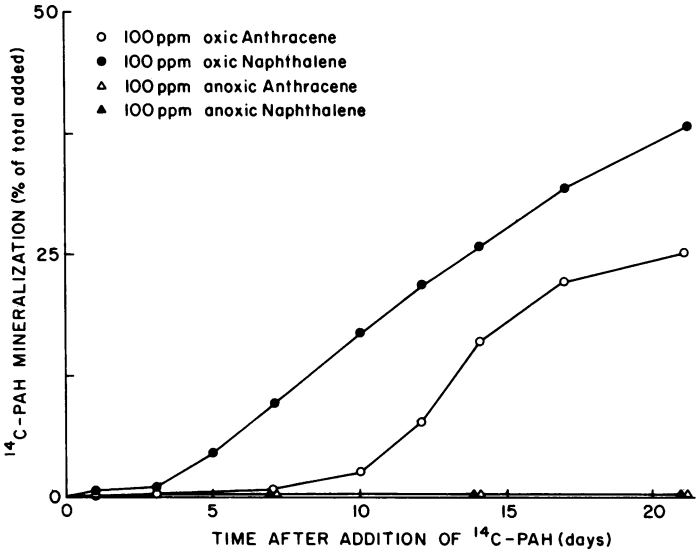

FIG. 1. Typical mineralization time series of [^{14}C]anthracene and -naphthalene by intertidal sediment slurries under oxic and anoxic conditions. All points ± standard deviation. n = two replicates.

amended with 100 ppm plus 10 μl of ^{14}C-labeled organics to a headspace containing 0, 10, 20, 40, 75, or 100% O_2. Headspace O_2 was regulated by gassing vials with nitrogen gas and readjusting to the desired O_2 concentration by withdrawing predetermined quantities of N_2 and adding an equivalent volume of O_2 with a plastic syringe. A similar experiment assessed the effects of low O_2 tensions on [^{14}C]anthracene and -naphthalene mineralization by sediments unacclimated or acclimated to 100 ppm of the two compounds for 14 and 7 days, respectively.

In the third O_2 experiment, triplicate 5-ml slurry incubations with anoxic sediment under anoxic conditions were amended with 100 ppm plus 10 μl of ^{14}C-labeled PAH. After 3 months under anoxic (N_2 headspace) conditions, incubations were converted to oxic conditions by gassing with compressed air. Gassing with air was periodically continued for 4 weeks, and $^{14}CO_2$ was trapped and quantified.

Effects of compound-related factors. Acclimation of microbial populations to compounds was carried out by adding 1 to 1,000 ppm of organic compounds to single-batch enrichments (100 ml) of 1:2-diluted sediment slurry. For time-dependent acclimation studies, triplicate subsamples were withdrawn at selected times and reamended with 200 ppm plus 10 μl of ^{14}C-labeled PAH. The effects of pre-exposure concentration on mineralization were assessed by preincubation with 0, 10, 100, or 1,000 ppm of organics and assaying by reamendment with 100 ppm plus 10 μl of ^{14}C-labeled PAH. The effects of reamendment concentration on mineralization were determined by preincubation with 100 ppm of organic and reamendment with 1, 10, 100, or 1,000 ppm of organic plus 10 μl of ^{14}C-labeled PAH.

Statistical analyses. All comparisons of rates and final amounts of various activities between treatments in experiments examining environmental and compound-specific effects on PAH mineralization and microbial populations were analyzed by single-classification analysis of variance. Significance levels were set at $P < 0.05$ or less for all analyses. Procedures for these analyses as well as all regressions, correlations, and transformations were obtained from Sokal and Rohlf (22) and Zar (30). For all tables and figures in which P values are not explicitly stated, the following convention was used: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

RESULTS

Degradation of naphthalene and anthracene. Figure 1 illustrates a typical mineralization time course of ^{14}C-labeled anthracene and naphthalene under oxic and anoxic conditions. Oxic [^{14}C]naphthalene mineralization proceeded after

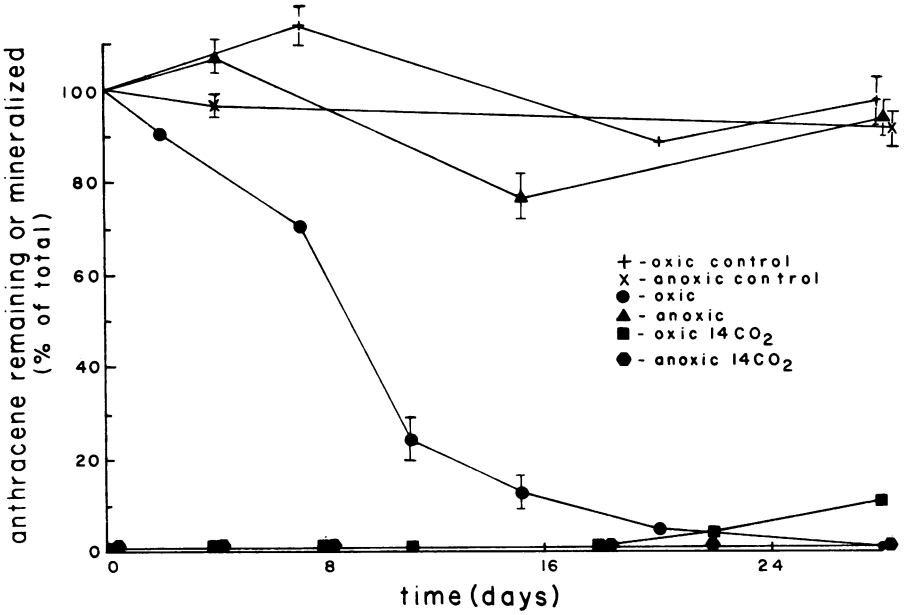

FIG. 2. Degradation (anthracene disappearance) and mineralization of 100 ppm of [^{14}C]anthracene by intertidal sediment slurries under oxic and anoxic conditions. All points ± standard deviation. n = three replicates.

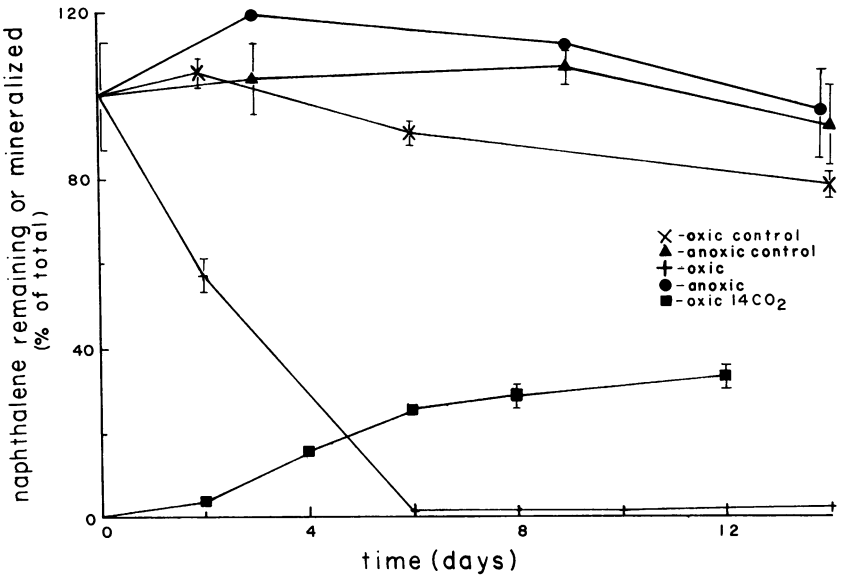

FIG. 3. Degradation (naphthalene disappearance) and mineralization of 100 ppm of [^{14}C]naphthalene by intertidal sediment slurries under oxic and anoxic conditions. All points ± standard deviation. n = three replicates.

a 1- to 3-day lag period as opposed to longer (8 days in this experiment, >20 days in others; e.g., see Fig. 2) lag periods exhibited by anthracene. Maximum rates of mineralization of both compounds under these conditions ranged from 1 to 4% per day for all experiments. Greater final amounts of naphthalene mineralized (32% per 21 days) were usually obtained compared with anthracene (22% per 21 days). Neither autoclaved controls (data not shown) nor anoxic sediments were ever found to produce ^{14}C-labeled mineralization products from [^{14}C]anthracene or -naphthalene.

Parent disappearance and mineralization of [^{14}C]anthracene and -naphthalene, respectively, under oxic and anoxic conditions are presented in Fig. 2 and 3. In these experiments, oxic anthracene degradation (parent disappearance) proceeded without lag and took 28 days for 99% of the compound to be transformed. $^{14}CO_2$ production lagged for 18 to 22 days, with 11% of the compound mineralized after 28 days. Neither anoxic nor autoclaved samples showed evidence of anthracene degradation by day 28.

Naphthalene showed similar, though more rapid, patterns of degradation when compared with anthracene (Fig. 3). Oxic degradation proceeded without lag and >99% of the parent compound was transformed by day 6. $^{14}CO_2$ production lagged for 2 days and 42% of the substrate was mineralized by day 12. Anoxic samples showed no differences in naphthalene concentrations from controls and were not significantly different from 100% by day 14. A slow apparent decrease in naphthalene in autoclaved controls may have been due to a small amount of volatilization and loss through the stopper which could not be prevented. Ampoulated autoclaved controls had 100% of the original naphthalene still present by experiment's end (data not shown).

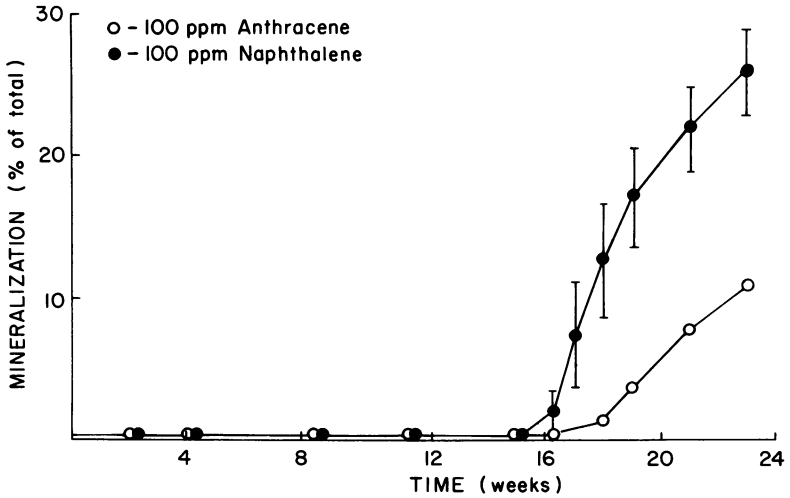

FIG. 4. Mineralization of 100 ppm of [^{14}C]anthracene and -naphthalene by intertidal sediment slurries under conditions of initial anoxia followed by oxic conditions (initiated at week 16). All points ± standard deviation. n = three replicates.

TABLE 1. Effects of temperature, nitrate, and glucose on 100-ppm [^{14}C]anthracene (A) and -naphthalene (N) mineralization by aerobic intertidal sediment slurries[a]

Treatment	Compound	Incubation time (days)	Maximum rate (%/day)	P[b]	Maximum amt (%)	P[b]
10°C (control)	A	28	0.8 (0.3)		8.7 (2.1)	
20°C	A	28	1.5 (0.8)	NS	11.1 (3.8)	NS
30°C	A	28	2.2 (0.1)	<0.01	16.2 (0.9)	<0.01
10°C (control)	N	14	1.4 (0.2)		8.0 (2.2)	
20°C	N	14	3.6 (0.3)	<0.001	31.1 (1.5)	<0.001
30°C	N	14	6.2 (0.5)	<0.001	34.9 (2.9)	<0.001
Control	A	28	1.5 (0.8)		11.1 (3.1)	
50 μM NO_3^-	A	28	1.8 (0.5)	NS	10.7 (2.1)	NS
500 μM NO_3^-	A	28	2.2 (0.1)	NS	20.4 (6.2)	NS
5 mM NO_3^-	A	28	3.0 (0.5)	NS	23.1 (2.4)	<0.01
Control	N	14	2.0 (0.2)		23.1 (2.3)	
50 μM NO_3^-	N	14	2.2 (0.1)	NS	25.4 (0.7)	NS
500 μM NO_3^-	N	14	2.1 (0.1)	NS	24.2 (2.4)	NS
5 mM NO_3^-	N	14	2.0 (0.1)	NS	22.1 (2.3)	NS
Preadaptational						
Control	A	14	1.5 (0.5)		7.0 (1.3)	
100 ppm of glucose	A	14	1.4 (0.5)	NS	8.6 (2.5)	NS
Control	N	13	3.4 (0.7)		30.2 (2.1)	
100 ppm of glucose	N	13	3.8 (0.7)	NS	30.4 (1.1)	NS
Postadaptational						
Control	A	14	3.1 (0.1)		29.8 (0.8)	
100 ppm of glucose	A	14	3.0 (0.1)	NS	29.3 (0.5)	NS
Control	N	7	5.0 (0.3)		21.6 (0.3)	
100 ppm of glucose	N	7	3.1 (0.1)	<0.001	23.8 (6.3)	NS

[a] See text for full description of treatments. Standard deviations are given in parentheses.
[b] All significance tests were performed relative to control values. NS, Not significant.

Although anoxic sediments were never found to mineralize [^{14}C]anthracene or -naphthalene, anoxic sediments which were converted to oxic conditions (air in headspace) readily mineralized both compounds (Fig. 4). After 16 weeks no mineralization products had been detected from anoxic sediments. However, 11.2 and 26.2% of 100 ppm of anthracene and naphthalene, respectively, were oxically mineralized after the same sediments were exposed to air for an additional 8 weeks.

Effects of environmental factors on anthracene and naphthalene mineralization. Table 1 lists maximum rates and amounts of [^{14}C]anthracene and -naphthalene mineralized under various treatments of temperature and nitrate and glucose enrichment. Significantly greater mineralization

TABLE 2. Effects of 0 to 100% headspace oxygen on mineralization of 100 ppm of [^{14}C]anthracene and -naphthalene by intertidal sediment slurries

Compound	Incubation time (days)	% O_2	Maximum rate (%/day)[a]	Maximum amt (%)[a]	Lag period (days)
Anthracene	20	0	0	0	
		10	0	0.2 (0)	
		20	0.2 (0.2)	1.4 (0.9)	17
		40	1.6 (0.4)	11.8 (1.2)	9
		75	1.1 (0.1)	7.8 (0.6)	13
		100	1.0 (0.4)	6.6 (2.3)	13
Naphthalene	14	0	0	0	
		10	3.8 (0.2)	33.4 (1.6)	2
		20	3.0 (1.2)	27.7 (3.5)	2
		40	4.3 (0.5)	23.5 (2.0)	2
		75	1.3 (0.2)	8.7 (0.6)	4
		100	0.8 (0.1)	5.5 (0.5)	4

[a] All values are ± standard error (in parentheses).

TABLE 3. Effects of oxygen on mineralization of 100 ppm of [^{14}C]anthracene and -naphthalene by intertidal sediment slurries under nonacclimated and acclimated (pre-exposed to 100 ppm of PAH) conditions

Expt	Incubation time (days)	% O_2	Maximum rate (%/day)[a]	P^b	Total at day 7 (%)[a]	P^b
2/21/84	21	0	0	<0.001	0.04 (0)	<0.001
Non-acclimated anthracene		2.5	0.03 (0.02)	<0.001	0.3 (0.1)	<0.001
		5	0.18 (0.04)	<0.001	1.3 (0.3)	<0.001
		7.5	0.6 (0.2)	<0.05	4.0 (0.8)	<0.05
		10	0.7 (0.02)	<0.05	5.1 (0.4)	<0.05
		20	1.2 (0.2)		7.5 (1.4)	
2/6/84	7	0	0.02 (0.01)	<0.001	0.1 (0.02)	<0.001
Acclimated anthracene		2.5	6.4 (0.7)	<0.01	38.7 (4.2)	<0.01
(10 days)		5	6.3 (1.10)	<0.01	42.3 (0.3)	<0.001
		7.5	8.1 (0.2)	<0.05	48.8 (0.6)	<0.01
		10	8.5 (0.3)	NS	51.3 (2.1)	NS
		20	8.9 (0.1)		52.6 (1.0)	
10/5/83	12	0	0 (0)	<0.001	0.1 (0)	<0.001
Non-acclimated naphthalene		2.5	1.8 (0.4)	<0.01	7.6 (2.0)	<0.01
		5	4.0 (0.5)	NS	19.6 (1.7)	NS
		7.5	4.0 (0.5)	NS	22.2 (2.4)	NS
		10	4.4 (0.1)		22.2 (1.2)	
2/27/84	7	2.5	0.9 (0.1)	<0.001	6.3 (0.7)	<0.01
Acclimated naphthalene		5	2.4 (0.5)	<0.01	15.8 (2.7)	<0.01
(8 days)		7.5	3.4 (0.1)	<0.01	22.4 (0.8)	<0.01
		10	4.4 (0.3)	NS	27.9 (1.6)	NS
		20	4.3 (0.2)		26.1 (0.9)	

[a] All values are ± standard deviation (in parentheses).
[b] Statistical comparisons (Student's t test) were performed relative to uppermost O_2 level values. NS, Not significant.

rates and amounts of [^{14}C]anthracene mineralized were observed at 30°C, but not at 20°C, compared with 10°C samples. A doubling and tripling in [^{14}C]anthracene mineralization rate occurred at 20 and 30°C, respectively, over 10°C incubations. Similarly, naphthalene mineralization rates increased 2.7 and 4.6 times over 10°C incubations at 20 and 30°C, respectively. Final amounts of both substrates mineralized underwent smaller increases than mineralization rates for the corresponding increases in temperature except for naphthalene between 10 and 20°C, which increased about fourfold.

None of the other treatments (except post-adaptational glucose treatment of naphthalene slurries and 5 mM NO_3^- treatment on total anthracene mineralization) resulted in significant alteration of anthracene or naphthalene mineralization rates or maximum amounts mineralized relative to controls. Increasing trends occurred in anthracene mineralization rate and maximum amount mineralized with increasing NO_3^- concentration.

Effects of oxygen. The maximum rate and amount of [^{14}C]anthracene mineralized were highest at 40% O_2 when sediments were exposed to 0 to 100% O_2 (Table 2). For naphthalene, the highest rate of mineralization occurred at 40% O_2, whereas the greatest amount mineralized was at 10% O_2.

[^{14}C]anthracene and -naphthalene mineralization rates as a function of O_2 concentration in slurries both acclimated and nonacclimated to the substrates are presented in Table 3. Rates and total amounts of [^{14}C]anthracene and -naphthalene mineralization increased with increasing O_2 concentration in all experiments, including unacclimated sediments as well as those pre-exposed to 100 ppm of anthracene and naphthalene;. The greatest increase in both rate and total amount of mineralization occurred between 0 and 5% O_2.

Lineweaver-Burke transformations of the data were performed to determine the maximum rates of PAH mineralization (V_{max}) as well as the half-saturation constants (K_s) and PAH turnover times (T_t) under oxygen limitation (Table 4). [^{14}C]anthracene mineralization rate did not exhibit saturation kinetics under nonacclimated conditions. Therefore, the rate of [^{14}C]anthracene mineralization at 20% O_2 was used instead of V_{max}. V_{max} was higher and turnover times were lower for slurries already acclimated to anthracene and naphthalene. Whereas anthracene turnover was much slower than naphthalene turnover under nonacclimated conditions, acclimation resulted in more rapid turnover of anthracene versus naphthalene. Comparison of K_s values

TABLE 4. Kinetic parameters of mineralization of [^{14}C]-anthracene and -naphthalene by salt marsh sediments as a function of headspace oxygen concentration, using the data of Table 3

Expt	V_{max} (%/day)	T_t (days)	K_s (% O_2)
2/21/84 Nonacclimated anthracene	1.2	85	NA
2/6/84 Acclimated anthracene	9.4	11	1.1
10/5/84 Nonacclimated naphthalene	4.9	21	1.7
2/27/84 Acclimated naphthalene	5.8	17	6.3

[a] V_{max}, Maximum PAH mineralization rate.
[b] T_t, PAH turnover time.
[c] K_s, Half-saturation constant for O_2. (percent headspace) NA, Not applicable to this experiment.

was only possible for naphthalene and values were lower under nonacclimated conditions. The K_s for anthracene under acclimated conditions was smaller than either naphthalene value.

Compound-related effects on mineralization. Figure 5 presents mineralization time courses of [^{14}C]anthracene and -naphthalene at 100-ppb, 5-ppm, and 100-ppm concentrations. Maximum rates and final amounts of substrates mineralized by 22 days were generally proportional to substrate concentration for both compounds. Maximum rates of mineralization averaged 0.7, 1.2, and 2.3% per day for anthracene and 2.7, 4.1, and 4.1% per day for naphthalene for 100-ppb, 5-ppm, and 100-ppm concentrations. In comparison, the absolute amounts of total anthracene mineralized were 6.7 ng, 260 ng, and 9.4 μg for anthracene and 62 ng, 1.2 μg, and 23 μg for naphthalene at 100 ppb, 5 ppm, and 100 ppm, respectively. The lag period for all incubations was apparently not concentration dependent.

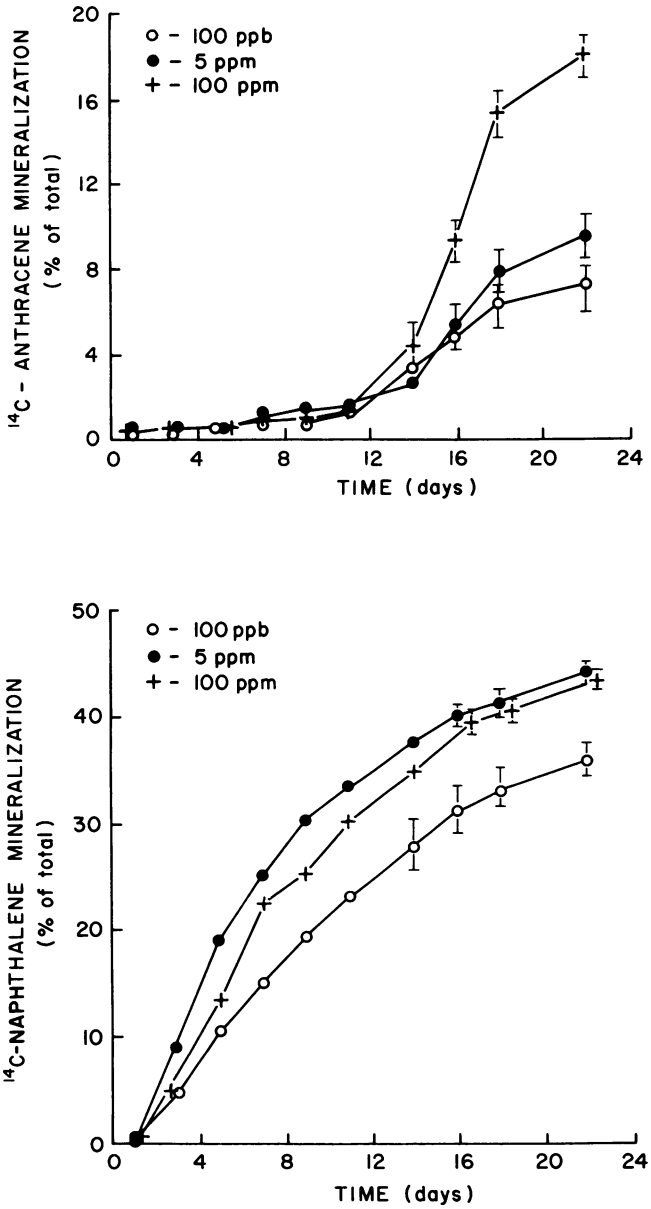

FIG. 5. Mineralization of 100 ppb, 5 ppm, and 100 ppm of [^{14}C]anthracene and -naphthalene by aerobic intertidal sediment slurries. All values ± standard deviation. n = three replicates.

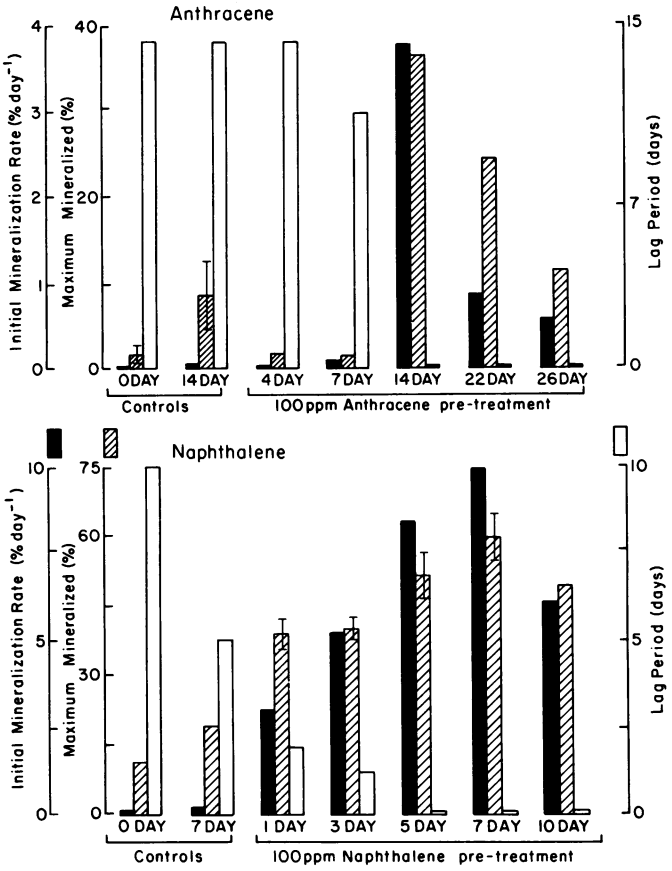

FIG. 6. Lag periods, initial rates, and total amounts of mineralization of [^{14}C]anthracene and -naphthalene by aerobic intertidal sediment slurries (100-ml batches) initially receiving 100 ppm of the respective compound. Subsamples were withdrawn from each batch at the indicated time intervals and redosed with 100 ppm of [^{14}C]anthracene or -naphthalene, and $^{14}CO_2$ was collected. All points ± standard deviation. n = three replicates.

The pre-exposure time dependence of PAH mineralization is shown in Fig. 6. Control samples assayed after 7 or 14 days showed slightly higher rates and total amounts of naphthalene or anthracene mineralization than day zero controls (possibly due to carbon limitation). However, these differences were small when compared with anthracene-pretreated sediments. Initial [^{14}C]anthracene mineralization rate increased from 0.01% per day after 4 days of pretreatment to a maximum of 3.9% per day after 14 days of pretreatment; maximum naphthalene rates were observed after 7 days (10% per day). The maximum amount of [^{14}C]anthracene mineralized also occurred after 14 days of preincubation (36.5% of the total added); maximum naphthalene mineralized was 59% after 7 days of pretreatment. All anthracene slurries pretreated for longer than 14 days, or naphthalene for longer than 5 days, showed no lag period.

The time dependence of acclimation to anthracene and naphthalene was also investigated by means of spread-plate experiments. Percentages of total numbers consisting of "organic pollutant-tolerant" bacteria were compared for control batches and those treated at time zero with 100 ppm

TABLE 5. Initial rates and final amounts of 100-ppm [^{14}C]-anthracene and -naphthalene mineralization by aerobic salt marsh sediment slurries after pre-exposure to the indicated concentrations of each compound

Compound	Concn (ppm)	Initial rate (%/day)[a]	Total amt (%)[a]	Lag (days)
Anthracene[b]	0	0.07 (0.003)	0.85 (0.41)	
	10	0.58 (0.07)**	5.0 (0.15)**	0
	100	0.88 (0.01)**	6.7 (0.07)**	0
	1,000	3.8 (0.33)***	25.6 (2.4)**	0
Naphthalene[c]	0	1.8 (0.23)	25.7 (4.8)	2
	10	1.2 (0.10)	29.7 (0.32)	2
	100	8.0 (0.04)***	48.0 (0.60)*	0
	1,000	10.3 (0.23)***	55.0 (0.90)**	0

[a] All values are ± standard error (in parentheses). Significant differences (by Student's *t* test) are expressed relative to O-ppm controls (see text).
[b] 10-day pre-exposures.
[c] 7-day pre-exposures.

of the organic compound. Time zero colony counts were performed on single spread plates only; therefore, no statistical comparisons were possible for these time points.

Numbers of total bacteria per milliliter of sediment slurry increased slightly in all batches over the course of the plating experiment (1 month) from 32×10^6 to 36×10^6 cells per ml of slurry. Relatively high percentages of the total bacteria from both controls and anthracene-treated batches were found to grow on anthracene-enriched plates at the beginning of the experiment (21 and 29%, respectively) when compared with naphthalene-enriched plates (2%). The maximum difference between control and anthracene-treated batches (16 and 43%, respectively) occurred after 2 weeks of preexposure. A similar maximum in naphthalene-tolerant bacteria from naphthalene-treated batches compared with controls also occurred at about 2 weeks (42 versus 10%, respectively).

Initial mineralization rate and total amounts of anthracene and naphthalene mineralized were a direct function of the pre-exposure concentration (Table 5). Initial rates were greatest at the highest exposure concentration of both anthracene and naphthalene (3.8 and 10% per day, respectively), as were total amounts mineralized (26 and 55%, respectively).

Different patterns of [^{14}C]anthracene and -naphthalene mineralization were observed when sediment slurries were pre-exposed to 100 ppm of the PAHs and re-exposed to 1 to 1,000 ppm supplemented with [^{14}C]anthracene or -naphthalene (Table 6). Although anthracene and naphthalene re-exposure concentrations ranged over three orders of magnitude, all concentrations (except 1 ppm of anthracene) elicited nearly identical [^{14}C]anthracene (7.5 to 7.7% per day) and [^{14}C]naphthalene (6.2 to 8.1% per day) rates of mineralization and total amounts of mineralization (46 to 47% and 40 to 49%, respectively). [^{14}C]anthracene mineralization at 1 ppm (6.0% per day and 37% final) was significantly lower than mineralization at all other re-exposure concentrations. Absolute rates and final amounts of anthracene and naphthalene mineralized ranged over three orders of magnitude (Table 6).

DISCUSSION

The present studies of the degradation and mineralization of the PAHs anthracene and naphthalene provide evidence for the possible fate and effects of these compounds in coastal sedimentary environments. Knowledge of the capacity of a sediment to degrade various classes of compounds as well as the acute and chronic effects of those compounds on the resident microbiota may enable predictions of short- and long-term compound/microbe interactions to be made.

The lack of anoxic anthracene and naphthalene degradation was not unexpected. Molecular oxygen has been found to be necessary for aromatic catabolism as it is directly incorporated into the aromatic ring structure (6, 13). It has been observed that monoaromatic benzoates can be degraded anaerobically by initial ring reduction followed by hydrolytic cleavage (9, 17, 24). However, similar mechanisms have not been reported for polyaromatic compound breakdown. No mineralization of the PAH anthracene or naphthalene was observed in reducing sediments of Louisiana salt marshes (8).

The present study confirms that [^{14}C]anthracene and -naphthalene mineralization is strongly dependent on the availability of oxygen in the sediments (Tables 2 to 4; Fig. 1 to 4). No anthracene or naphthalene was found to be mineralized in sediments which were incubated without oxygen. In most cases in which incubations took place at 0 to 20% oxygen (in headspace), both mineralization rates and total amounts of anthracene and naphthalene mineralized increased with increasing oxygen.

The discrepancy between the total disappearance of parent compound and only partial recovery as inorganic carbon end product is noteworthy. If the amount of parent anthracene is considered negligible by day 28, whereas only 11% of the ^{14}C-labeled substrate had been mineralized by that time, the balance, or up to 89% of the initial anthracene, must have been distributed among cellular pools and intermediate metabolites. Similarly, 58% of the original naphthalene under oxic conditions may have ultimately been distributed in these pools (Fig. 3).

The long apparent lag period observed in oxic [^{14}C]anthracene mineralization may be an artifact due to the specific ^{14}C label. Anthracene is labeled on C-9 (inner ring) and this ^{14}C label would be expected to be one of the last to be released as CO_2 (12). This is further supported by the immediate decrease in parent anthracene while $^{14}CO_2$ production lagged up to 21 days (Fig. 2).

Analysis of the kinetics of PAH mineralization as a function of oxygen concentration illustrates both the impor-

TABLE 6. Initial rates and final amounts (both relative and absolute) of 1-to 1,000-ppm [^{14}C]-anthracene and -naphthalene mineralization by aerobic salt marsh sediment slurries after pre-exposure to 100 ppm of the compounds

Compound	Concn (ppm)	Initial rate (%/day)[a]	Final amt (%)[a]
Anthracene[b]	1	5.96 (0.20)*	37.3 (1.73)*
	10	7.50 (0.15)	46.0 (0.94)
	100	7.66 (0.06)	46.9 (0.35)
	1,000	7.69 (0.34)	46.8 (1.97)
Naphthalene[c]	1	7.68 (0.08)	46.7 (0.43)
	10	8.06 (0.27)	49.6 (0.63)
	100	7.60 (0.25)	46.6 (1.41)
	1,000	6.24 (0.78)	39.7 (4.05)

[a] All values are ± standard deviation (in parentheses). Significant differences were determined through single-classification analysis of variance (see text).
[b] 14-day pre-exposures.
[c] 7-day pre-exposures.

tance of oxygen and distinctions in the anthracene- and naphthalene-oxidizing systems (Table 4). First, maximum potential rates of mineralization of both PAHs were higher and turnover times were lower after sediments were acclimated to the compounds. The K_s values for O_2 with naphthalene were lower in nonacclimated versus acclimated sediments, whereas the K_s for O_2 in the anthracene experiment was lower than either naphthalene value. The distinct characteristics of the two PAH-oxidizing systems were also illustrated by the optimum naphthalene and anthracene mineralizations at 40 and 10% O_2, respectively (over a range of 1 to 100%). It should be noted that surface sediments have been found to contain oxygen at supersaturating levels due to benthic photosynthetic oxygen production (18). Sediments therefore have the potential to be exposed to these optimal levels of oxygen on at least a temporary basis.

These results agree well with those of DeLaune et al. (8), who found [^{14}C]-naphthalene mineralization to be directly related to sediment redox potential (or Eh), which in turn is largely controlled by oxygen availability. Hambrick et al. (11) found 0 and 35% of [^{14}C]naphthalene mineralized at −250 and +250 mV, respectively, after 35 days. This has several implications for both the fate of aromatic compounds in systems of variable redox and their long-term effects on resident microbiota.

In addition to temperature and oxygen, the factors affecting [^{14}C]anthracene and -naphthalene mineralization most were compound-related factors. Suggested mechanisms for enhanced mineralization capacity of microbes include enzyme induction, population selection, or plasmid transfer (28). Evidence for the first two of these was provided in plating experiments, time series of degradation, and concentration effects experiments (Tables 5 and 6; Fig. 5 and 6). The buildup and decay of anthracene-degrading populations was found to occur with a maximum by about 2 weeks after the initial acute addition of anthracene. This paralleled the effect of preincubation time on initial rates (Fig. 6). For naphthalene, a slightly earlier maximum occurred between 1 and 2 weeks. Time dependence of adaptation to mineralization of pentachlorophenol by terrestial soil systems (15) and *p*-nitrophenol by freshwater sediments (23) has also been observed.

Initial addition of higher concentrations of PAHs resulted in greater percentages of ^{14}C-labeled PAHs mineralized (Fig. 5). Boethling and Alexander (4) found threshold concentrations of many compounds below which no mineralization occurred. Results presented here showed significant PAH mineralization even at the lowest tested concentrations (100 ppb). However, thresholds may exist for anthracene and naphthalene at lower (<100 ppb) concentrations.

A quantitatively greater adaptational response (mineralization) was exhibited by sediments pre-exposed to higher concentrations of the two PAHs. An adaptational threshold appeared to exist below 10-ppm pre-exposures for naphthalene but not for anthracene (Table 5). Other studies have found the exposure history of a site to be important to its capacity to degrade aromatic pollutants. Herbes and Schwall (12) noted anthracene and naphthalene turnover to be 3,000 and 125,000 times greater, respectively, in oil-polluted stream sediments than in nonpolluted ones. Similar enhancement of naphthalene and phenanthrene mineralization was noted in freshwater microcosm sediments which were pre-exposed to synthetic oil for 1 month (21).

Sediments effectively utilized a wide concentration range of PAHs after pre-exposure to a single concentration (100 ppm; Table 6), and the mineralization of further inputs of PAHs occurred at relative rates (percent per day) independent of compound concentration. The concentration independence of mineralization after pre-exposure to 100 ppm of PAHs suggests maximum development of PAH-degrading populations or induction of enzyme systems capable of "buffering" the system against further PAH inputs and rapidly degrading PAHs. It must be noted that residual anthracene or naphthalene may have remained in acclimated slurries after the acclimation periods (and was not quantified). Any residual PAH would have resulted in differences in the specific activities of the compounds. However, based on high-pressure liquid chromatography extractions of sediments, it appears that any residual PAH after the acclimation period would result in relatively minor errors in the percentages mineralized.

Temporal and spatial variability in the oxidation state of coastal marine sediments would likely result in intermediate degrees of anthracene and naphthalene degradation. As was illustrated by the anoxic/oxic conversion experiment, which was initiated with strictly anoxic sediment (Fig. 4), facultative PAH-degrading aerobes apparently exist under anoxic conditions. When oxygen was made available, these heterotrophs readily oxidized the PAHs present. Anoxic sediments which are periodically oxidized through macrofaunal bioturbation (10), physical mixing, or benthic photosynthesis (18) may eliminate PAHs and mitigate their toxic effects more effectively than those which are strictly anoxic, but less effectively than fully oxidized surface sediments. These differences in removal rate between distinct sediment types may also account for the varying degrees of PAH toxicity to sediment bacteria which have been observed (3, 14; J. Slater and D. G. Capone, *in* I. Duedall, ed., *Proceedings: Fourth Ocean Dumping Symposium*, in press).

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