MICROBIAL ECOLOGY

Microb Ecol (1999) 37:152–162

Effect of Crude Oil and Chemical Additives on Metabolic Activity of Mixed Microbial Populations in Fresh Marsh Soils

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Received: 27 April 1998; Accepted: 15 July 1998

Abstract

Hydrocarbons increase abundance of hydrocarbon-degrading microorganisms, but also decrease microbial diversity. This could disrupt ecosystem dynamics by altering soil organic matter mineralization and resultant nutrient remineralization rates. Crude oil, which is known to contain toxins and reduce microbial diversity, was hypothesized to reduce gross metabolic activity of mixed microbial populations in wetland soils. Soil respiration and Eh were compared, for 6 months, among microcosms containing marsh soils that differed in soil organic matter (*Panicum hemitomon* Shult. or *Sagittaria lancifolia* L. dominated marshes), crude oil (Arabian crude, Louisiana crude, or no oil), and additives (a cleaner, a dispersant, fertilizer, or no additive). No treatment slowed activity; instead, Louisiana plus fertilizer and all Arabian treatments temporarily accelerated activity. Additional C respired from oiled microcosms exceeded C added as crude oil by 1.4 to 3.5 times. Thus, much additional C originated from soil organic matter rather than crude oil. Crude oils temporarily lowered soil Eh, which is consistent with accelerated metabolism and demand for electron acceptors. The lack of inhibition observed at the community level does not necessarily indicate an absence of toxicity. Instead, tolerant species with metabolic versatility probably maintained activity. Stimulation probably resulted from removal of micronutrient limitation, rather than removal of grazing pressure or macronutrient limitation. Regardless, accelerated soil organic matter mineralization surely accelerated nutrient remineralization. This might explain some reports of crude oil stimulating plant growth. These results are not inconsistent with theoretical and experimental conclusions regarding effects of biodiversity on ecosystem stability and productivity, nor are they inconsistent with conclusions that crude oils contain components that are toxic to microbes, vegetation, and fauna. However, these data do indicate that crude oils also contain components that temporarily stimulate metabolic activity of surviving microbes.

Introduction

Perhaps the most important role of soil microorganisms is their participation in nutrient remineralization [71]. The importance of soil organic N and P mineralization in providing labile nutrients to plants is well established [67], and the only nutrients generally available to plants are those released during the mineralization of soil organic matter that

exceed the needs of the decomposers [31]. Thus, the regeneration of assimilable nutrients is key to understanding the regulation of ecosystem function [58]. Mineralization processes deserve greatest priority when evaluating effects of pollutants on soil microorganisms [24]. Nutrient remineralization strongly governs productivity in wetlands, which provide more services, per unit area, than other terrestrial ecosystems [13]. Nutrient release is slower, and less variable, in wetlands than in other terrestrial ecosystems because of adequate moisture and large pools of organic matter. Consequently, wetland soil organic matter mineralization, and resulting nutrient remineralization, are decoupled from primary production [36].

Microbial mineralization of soil organic matter also influences wetland plant production by creating soil anoxia, which is commonly measured as Eh [25]. Oxia prevails a few millimeters near the surface and plant roots. Away from oxygen sources, however, lie zones of archaea and facultative and obligate anaerobic bacteria using less efficient electron acceptors and decreasing Eh. However, anoxia can occur regularly even at the surface and near plant roots, because the aerobic zone expands and shrinks within minutes in response to O_2 production and consumption [53]. Changes in soil water chemistry coincide with changes in soil Eh [26], and soil Eh has been used as indicator of root stress on fresh, brackish, and saline marsh vegetation [9, 20, 41].

Previous descriptions of community structure in wetlands soils [23, 75] may be incomplete or biased because of the inability to culture viable microbes [72, 73]. Recently applied culture-independent techniques are already indicating a greater diversity than previously believed. For instance, methanogens are more common in sulfate-rich environments than previously thought [44]. Most soil microbes are archaea and facultative/obligate anaerobic bacteria because of anoxia. Individual species cannot completely mineralize soil organic matter to CO_2, but species with different metabolic capabilities and limitations form consortia in which soil organic matter is mineralized to CO_2 anaerobically [46]. Consortia grow, reproduce, and recycle nutrients more effectively and efficiently than individual populations [53]. The primary grazers on archaea and bacteria are likely to be ciliates and nematodes that can dive into anaerobic areas to feed [27]. Grazing regulates and contributes to rapid mineralization of soil organic matter by maintaining microbial productivity and preventing senescence [36]. Grazing also transfers energy harvested by soil archaea and bacteria to higher trophic levels.

It has long been recognized that crude oil increases activity and abundance of hydrocarbon-degrading bacteria, fungi, and yeast [3]. Data are accumulating to indicate that some wetland plant species tolerate fouling with some crude oils [21, 39, 55, 56, 62]. Despite the prevalence of tolerant species, crude oil also apparently contains toxic components that reduce diversity of soil bacteria [6], water column bacteria [4, 34], benthic invertebrates [6, 60], and emergent plant communities [39]. However, there are no studies of the effects of crude oil on wetland soil community-level microbial functions. It is assumed that oil spills degrade ecosystem integrity and function because lower diversity disrupts the tight coupling and interdependence within and among consortia, and between consortia and grazers.

The effect that crude oil has on wetlands varies with human response. Physical removal of oil from wetlands is unwise because even human foot traffic causes severe and long-lasting damage [5, 32, 51]. No response, as is recommended for some rocky shorelines [42, 51], is an option, because oil can evaporate and naturally degrade in wetlands [19, 30]. Three types of chemicals additives can be used to speed oil disappearance: dispersants, cleaners, and nutrients. Dispersants are added to floating oil in deep waters. Dispersant use may increase because new dispersants are less toxic than older dispersants [14]. Although it is highly unlikely, marshes could be exposed to dispersed oil. Cleaners are used after oil has fouled a structure at the water/shore interface. Cleaners wash oil from surfaces, such as rocks and vegetation, back into the water without dispersion where it can be collected [28]. A cleaner prevented mortality of fouled *Rhizophora mangle* L. and *Spartina alterniflora* Loisel in greenhouse experiments [56, 68]. It is, therefore, possible that marshes could be exposed to mixtures of oil and cleaner. Nutrients can be added to floating oil or to oiled marshes to accelerate degradation of the oil. Nutrients are the only additive that can be applied on a wide-scale basis to marshes that have already been fouled with oil. It is possible that some chemical additives mitigate impacts of crude oil on soil microbial activity, whereas other additives exacerbate impacts.

The preceding discussion indicates that the effect of crude oil and chemical additives on microbial activity in wetland soils needs to be better understood. Crude oils, which are known to reduce microbial diversity [4, 6, 7, 60], were hypothesized to slow gross metabolic activity of mixed microbial populations in marsh soil. Respiration and soil Eh were compared among microcosms containing fresh marsh soils that differed in dominant plant species (*Panicum hemitomon* Schult. or *Sagittaria lancifolia* L.), crude oil (Arabian crude,

Louisiana crude, or no oil), and clean-up strategy (a cleaner, a dispersant, nutrient addition, or no additive). Respiration was measured because C emissions provide the best index of gross metabolic activity of mixed microbial populations in soil [66]. Furthermore, degradation of soil organic matter is highly sensitive to pollutants [24] and regulates ecosystem dynamics by making nutrients available for uptake [31, 58, 65, 71]. Soil from fresh marshes were studied because of a lack of information, despite their extensive exposure to wells, pipelines, and navigation in coastal Louisiana (where there are approximately 301,500 ha of *P. hemitomon* dominated marshes and 172,000 ha of *S. lancifolia* dominated marshes [12]). Two crude oils were used because crude oils differ in chemical makeup, toxicity, and metal content. For instance, Arabian and Louisiana crudes have similar density, but differ in solubility, N, and S content (Table 1).

Methods

Briefly, 144 microcosms were created by placing soils in flasks. Each flask was subjected to one of three oiling scenarios: Arabian Crude, Louisiana Crude, or no oil. Each flask was also subjected to one of four clean-up scenarios: no additive, dispersant addition, cleaner addition, or fertilizer addition. Thus, there were 12 treatment combinations. Soil respiration and soil Eh were monitored for 6 months following treatment.

Bulk soil samples and cores were collected from three marshes in coastal Louisiana, at ~1 mon intervals, during August and September 1994. The marshes were located >100 km apart. One marsh was near Lake Misere in Cameron Parish, one was near Lake Salvador in St. Charles Parish, and one was adjacent to the Tchefuncte River in St. Tammany Parish. Bulk samples were used to create microcosms; field conditions were characterized with cores.

Within each marsh, two 15-cm dia cores were collected from a area dominated by *P. hemitomon,* and two from another area dominated by *S. lancifolia.* They were returned to the lab and used to characterize organic matter content, moisture content, and soil bulk density within the upper 15 cm of field soils.

Within each marsh, separate bulk samples were collected from *P. hemitomon* dominated areas and from *S. lancifolia* dominated areas. Bulk soil was collected from the upper 30 cm, placed in covered, plastic tubs, and returned to the lab. Tubs were wrapped with a foil blanket to prevent temperature increases during transport back to the lab. Each bulk sample was homogenized by manually stirring and cutting with a serrated knife; large tubers of *S. lancifolia* were removed. All soils converted from firm peats to fluid pastes following destruction of the living root network even though no water was added to the bulk soil samples. After the soils were allowed to equilibrate for 1 week, ~300 ml of soil was placed in clean, preweighed and numbered, 500 ml Erlenmeyer flasks. They were then reweighed to determine the mass of soil paste added. Soils were allowed to equilibrate at least another 7 days before treatments were added; during this time the pastes separated into floating soil mats and underlying water, which is typical of Louisiana fresh marshes [61]. Forty-eight flasks from each area were prepared for incubation; thus, there were 144 flasks total.

Three 50-ml subsamples of each soil paste were placed in preweighed containers, weighed wet, dried at 100°C, and reweighed to determine bulk density of microcosm soil. Organic matter content of the samples was determined on a subsample of the dried samples, via combustion at 400°C for 12 h [15].

Arabian Crude oil (100 ml), Louisiana Crude oil (100 ml), dispersed oil (100 ml oil and 20 ml COREXIT 9550), and cleaned oil (100 ml oil and 20 ml COREXIT 9580) were weathered in 1 L beakers containing 300 ml of deionized water. Each beaker was continuously stirred under a fume hood overnight (16 h). This degree of weathering is mild relative to that when ultraviolet light and heat are applied. After weathering, oil and water were separated with a separatory funnel and stored in amber glass jars until they were applied to the microcosms. Water and oil fractions were separated so the oil/water ratio added to microcosms could be precisely replicated.

Two ml of oil fraction (1.8 g) and 6 ml (6 g) of the water fraction were added to the appropriate microcosms. This oil:water ratio was used to maintain the 1:3 ratio of weathering; this volume of oil was added to microcosms because preliminary trials indicated that this amount of oil covered approximately 75% of the surface area in similar flasks containing similar amounts of soil. The amount of oil used was equivalent to approximately 3.8 L/m^2. The fertilizer solution used was a 0.09 M $(NH_4)_2HPO_4$ solution prepared from stock chemicals; 10 ml was added to appropriate flasks. This application rate was equivalent to 4.1 g N/m^2 and 4.5 g P/m^2. For microcosms receiving cleaner or dispersant but no oil, 0.4 ml of chemical in 10 ml of deionized water was added to each flask.

The microcosms were kept uncovered and the soil shallowly flooded. It was not possible to flood the soil with more than a centimeter or two of water, because the soil floated. Deionized water was added to the flasks when the soil surface dropped below the original soil level; this allowed the soil surface to become occasionally exposed to air but never dry. Floating marshes are common in Louisiana [61], and two of the marshes sampled were buoyant. The microcosms behaved similarly to the floating marshes: They rose and fell with water levels, but remained saturated and generally had a centimeter or two of water on the surface.

Table 1. Characteristics of Arabian and Louisiana crude oils[a]

Characteristic	Arabian crude	Louisiana crude
API gravity at 60 F	33.4	35.8
% N	0.09	0.28
% S	1.78	0.23
Aqueous solubility (mg/L)	18.9	37.9

[a] From unpublished data available upon request from Environment Canada, Environmental Protection Directorate, River Road Environmental Technology Centre, Ottawa, Ontario.

The best and most easily measured index of gross metabolic activity of mixed microbial populations in soil is CO_2 evolution [66]. However, CH_4 is also an important avenue of C emission in wetland soils lacking sulfate; thus, both were measured. Carbon dioxide and CH_4 emissions were measured at roughly 1, 3, 7, 14, 28, 84, and 180 days after treatments were applied. These are, therefore, measurements of gross metabolic activity of the microbial community in the microcosms. Community is defined here as all of the organisms that occupy a particular site [1]. Frequent sampling during the first 30 days was intended to detect reversible effects; sampling beyond 30 days was intended to detect persistent effects [24]. Flasks were temporarily sealed with a rubber stopper equipped with a rubber septum, and air samples (0.5 ml) were collected with a gas syringe. The sample was immediately injected into a gas chromatograph; CO_2 and CH_4 were detected with flame ionization. Carbon dioxide was converted to methane with a methanizer heated to 300°C. Commercially available reference gases and a peak analysis software (EZ Chrom, Chromatography Data System, Scientific Software, Inc., San Ramon, CA) were used to calculate concentrations of CO_2 and CH_4 in samples. A second air sample was collected >90 min after the first sample, and the change in concentration between the two measurements was calculated. Flasks were then unsealed. Emission rates were calculated from the change in concentration, the time elapsed between the measurements, and the head space volume in the flask. Release of CH_4 by bubbles was sometimes indicated by extremely high CH_4 accumulations during the incubation. Those observations were retained, even though they decreased statistical power. Lacking sterile controls, it is possible that some of the C emitted from the soil resulted from abiotic CO_2 or CH_4 flux. Unfortunately, there are no data quantifying abiotic CO_2 or CH_4 flux from crude oil or saturated organic soils. However, given the lack of carbonate sediments, such as limestone and dolomite, the potential for abiotic CO_2 and CH_4 emissions is probably negligible. Sterile controls are generally used to assess the biodegradation potential of compounds, but are not generally used to assess respiration or gross metabolic activity of mixed soil populations.

Redox potential was measured in microcosms with duplicate bright platinum electrodes [25], at the soil surface and at 2.5 cm below the surface, throughout the incubation. Eh was measured only at the surface, where changes in the depth of oxic soil would be most detectable, and disturbance would be minimized. It was measured before treatment, and up to 112 d afterwards.

The sum of CO_2 and CH_4 emissions, expressed in terms of C emissions (g day^{-1} $microcosm^{-1}$), was used to measure gross metabolic activity (hereinafter referred to as activity). These data were analyzed as a $2 \times 3 \times 4$ factorial (plant type by oil treatment by clean-up), with blocking on site and measures repeated over time [64]; 983 observations were analyzed. The log transformation was used to normalize activity [64]. Effects with $P < 0.05$ were considered statistically significant; insignificant terms were pooled into the appropriate error term to improve error estimates. Emission rates of CO_2 and CH_4 were similarly analyzed. Carbon dioxide data were transformed with the (log +1), and CH_4 data were log-transformed, to improve normality [64]; 983 observations ea of CO_2 and CH_4 were analyzed. The percentage of C emissions accounted for by CH_4 was similarly analyzed. Zero estimates were set to 0.0001, and data were log-transformed, to improve normality [64]; 983 observations were analyzed. The percentage of C emissions accounted for by CH_4 was analyzed, because CH_4 emissions represent energy not captured by methanotrophs (methane oxidizing bacteria). This indicates less energy available to grazers and higher trophic levels.

Cumulative C emission from each microcosm throughout the 180-d experiment (g $microcosm^{-1}$) was compared to C additions from crude oil. It was assumed that the C emission rates measured on any given day were representative of rates from midway between the prior sampling day and that day to midway between that day and the subsequent sampling day. For instance, the measurements on day 3 were assumed representative of emissions from day 2 until day 5, and the measurement on day 84 was assumed representative of emission from day 56 until day 132. Least-square means [64] were used for this estimate; data from 119 flasks were used for this estimate, because of missing observations on some days from some flasks. Carbon added as crude oil was estimated to be 1.4 g C (given 1.8 g crude oil and assuming 80% C).

Soil Eh data were analyzed as a split plot (depth) with repeated measures (time) [64], using Proc GLM of SAS; 2,301 observations were analyzed. Eh data did not require transformation to achieve normality. Effects with a $P < 0.05$ were considered statistically significant; insignificant terms were pooled into the appropriate error term to improve error estimate.

Results

Organic matter content in the microcosm soils ranged from 41 to 90%; it averaged 52% in the *S. lancifolia* soil and 66% in the *P. hemitomon* soils. Bulk density was 5× greater in the microcosm soils than in the field soils (Table 2). Moisture content was extremely variable. For *S. lancifolia* marsh, it was greater in field soils than in microcosms; for *P. hemitomon* marsh, it was greater in microcosm soils than in field soils (Table 2).

Significant differences were detected in activity among the oil treatments over time ($F_{12,821} = 13.78$, $P = 0.0001$). Activity was initially similar among the 3 treatments, but differences appeared after adding treatments (Fig. 1). Differences persisted for at least 3 months, but were not found among the oiled and unoiled microcosms 6 months after crude oil was added (Fig. 1). The effects of the different crude oils and additives on activity are more easily interpreted by examining mean activity averaged over all dates (which also varied among the 12 oil and clean-up combinations) ($F_{6,124} = 8.478$, $P < 0.05$). Post-ANOVA comparisons with least-square means indicated that no treatment had slower mean activity than untreated microcosms (Fig. 2).

Table 2. Bulk density and percent water of field soils in the upper 15 cm of the marsh, and in experimental soils made from bulk samples obtained from the same depth at 3 *Sagittaria lancifolia* marshes and 3 *Panicum hemitomon* marshes in coastal Louisiana

Source marsh	Bulk density (g/cm^3)		Percent water	
	Field	Microcosm	Field	Microcosm
S. lancifolia marshes				
Lake Misere	0.014	0.059	126	143
Lake Salvador	0.016	0.128	112	82
Tchefuncte River	0.032	0.126	70	82
Average of *S. lancifolia*	0.021	0.106	103	102
P. hemitomon marshes				
Lake Misere	0.014	0.074	83	138
Lake Salvador	0.012	0.042	142	236
Tchefuncte River	0.021	0.152	85	69
Average of *P. hemitomon*	0.016	0.089	103	148

However, mean activity was faster in microcosms containing Louisiana crude oil treated with fertilizer, and in all microcosms containing Arabian crude oil, than in untreated microcosms and other treatments (Fig. 2). Mean activity was 1.4 times faster in *P. hemitomon* soils than in *S. lancifolia* ($F_{1,118} = 13.43$, $P = 0.0004$), but no difference was detected between the marsh types in the way they responded to the oil treatments ($F_{2,118} = 0.34$, $P = 0.7121$) or clean-up treatments ($F_{3,118} = 0.61$, $P = 0.6100$).

Emissions of CO_2 varied in a manner similar to activity (Figs. 1, 3); furthermore, CO_2 and activity were correlated ($R = 0.5595$, $n = 983$, $P = 0.0001$). Therefore, CO_2 emissions are not considered independently further. Carbon emitted as CH_4 also varied in a manner similar to activity, and emissions and activity were correlated ($R = 0.9212$, $n = 983$, $P = 0.0001$). Methane emissions are not independently considered further, except to note that increases in CH_4 emissions did not coincide with slower activity (Figs. 1, 4).

The percent of C emissions accounted for by CH_4 differed among the oil treatments over time ($F_{12,797} = 5.63$, $P = 0.0001$) and among the clean-up treatments over time ($F_{18,797} = 2.96$, $P = 0.0001$). The primary trend evident was

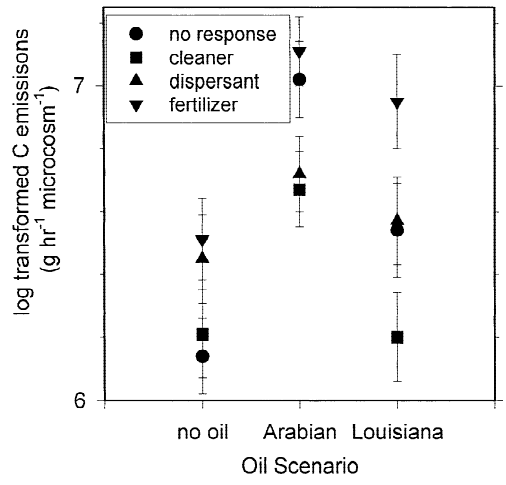

Fig. 2. Gross metabolic activity (g C h^{-1}) in flasks containing fresh marsh soil exposed to crude oils and additives, averaged over 7 observations during a 6-month incubation (least-square means and least-square standard errors).

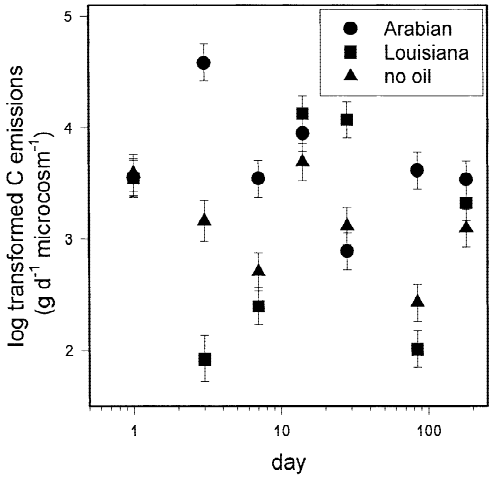

Fig. 1. Gross metabolic activity (g C h^{-1}) in flasks containing fresh marsh soil (microcosm) at different times, after exposure to crude oils and additives (least-square means and least-square standard errors).

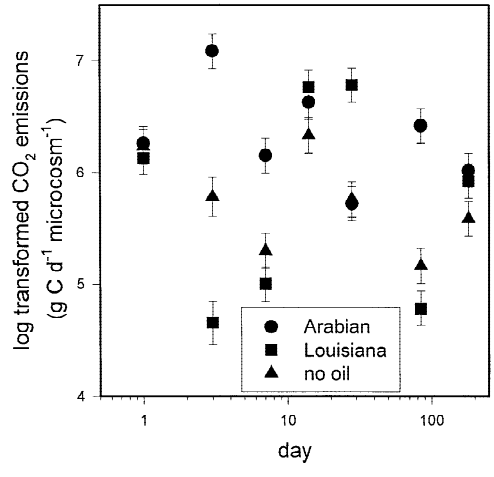

Fig. 3. Carbon dioxide emissions (g C h^{-1}) from flasks containing fresh marsh soil at different times, after exposure to crude oils and additives (least-square means and least-square standard errors).

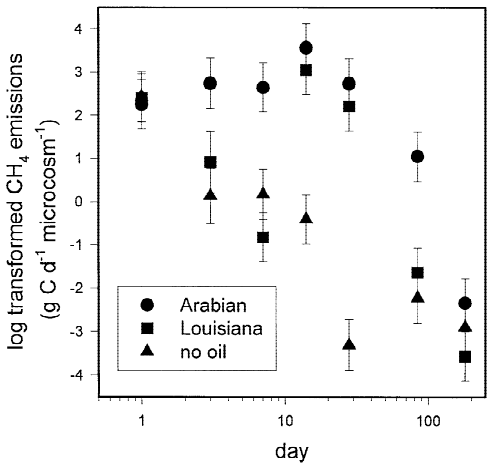

Fig. 4. Methane emissions (g C h^{-1}) from flasks containing fresh marsh soil at different times after exposure to crude oils and additives (least-quare means and least-square standard errors).

a decline in the proportion of C emitted as CH_4 over the 6-month incubation, but methane also appeared less important in untreated microcosms than in oiled ones (Fig. 5). Averaged over all sampling dates, methanogenic carbon accounted for 11% of C emissions. Post-ANOVA comparisons indicated that methane accounted for a greater percentage of C emissions in *P. hemitomon* soil, where activity was faster, than in *S. lancifolia* soil, with the following exception: Methanogenic C was greater in *S. lancifolia* soils treated with Arabian crude oil than in other *S. lancifolia* soils, and was, instead, similar to that in *P. hemitomon* soils.

Comparison of C added as crude oil to C emissions

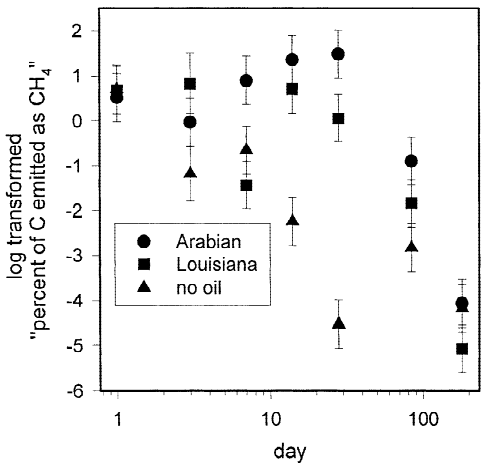

Fig. 5. Percent of carbon emitted as methane (least-square means and least-square standard errors) from marsh soil at different times after exposure to different crude oils and additives.

throughout the 6-month incubation indicated that too little crude oil was added to account for the entire increase in C emissions (Table 3). The amount by which C from oiled microcosms exceeded C from unoiled microcosms, 2.0 and 4.9 g from Louisiana and Arabian treatments respectively, exceeded the 1.4 g of C added as crude oil by 1.4 to 3.5 times. Less than one-half of the increase in C emissions could have arisen from microbial consumption of crude oil, even if all of the oil was consumed by the end of the incubations (15–20% remained, unpublished data). Much of the increase in C emissions, therefore, arose from microbial consumption of soil organic matter.

Eh was roughly 50 mV higher at the surface than 2.5 cm below the surface ($F_{1,84} = 117.58$, $P = 0.0001$). The continual methane emissions indicate that anoxia was present in the microcosms, but lay deeper than 2.5 cm below the surface. Eh was roughly 30 mV lower ($F_{1,106} = 6.43$, $P = 0.0127$) in *P. hemitomon* soil, where activity was faster and methane emissions more important, than in *S. lancifolia* soil.

Eh varied over time among the oil and clean-up treatments ($F_{54,1788} = 2.25$, $P = 0.0001$). Eh appeared to differ among treatments only in the first month of incubation. Average Eh over the four observations in the first 35 days of the incubations indicated that crude oils and additives lowered Eh, relative to the controls (Fig. 6). No differences were apparent among the treatments later in the incubations. Average Eh was near 350 mV when the treatments were added, and near 500 mV when the incubations ended.

Discussion

Some parameters differed between field and microcosm, but important patterns and processes were maintained. Differences in water content and bulk density between soils likely resulted from degassing of the soils and below-ground root material during homogenization. The greater bulk density of

Table 3. Carbon additions and emissions from flasks of marsh soil to which crude oil was added and emissions then measured over 6 months[a]

Treatment	Oil-C added (g)	C emitted (g)
No oil	0.0	5.8 (1.1)
Louisiana Crude	1.4	7.8 (1.3)
Arabian Crude	1.4	10.7 (1.0)

[a] Carbon added was estimated from the mass of crude oil added (1.8 g), assuming that crude oil is 80% C. Carbon emitted was estimated from least-square-means (least-square standard errors) of CO_2 and CH_4 emissions, measured 7 times throughout the incubation.

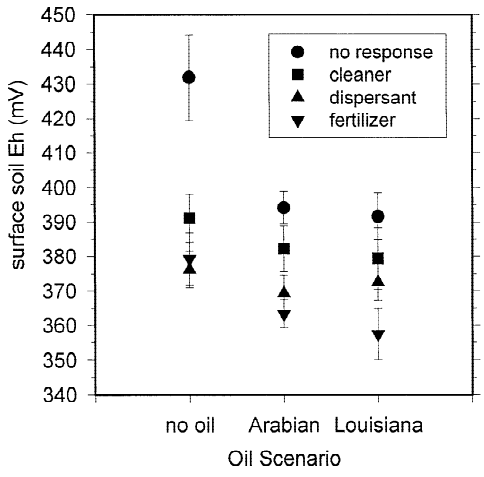

Fig. 6. Soil Eh (mV) averaged from 0.5 and 2.5 cm below the soil surface in flasks containing fresh marsh soil exposed to crude oils and additives, averaged over 4 observations during the first 35 days of a 6-month incubation. Error bars are standard errors; sample sizes range from 55 to 72 observations.

the microcosms, combined with a lack of living roots, probably decreased the thickness of the surface oxidized zone, relative to field conditions. The resulting smaller volume of oxidized soil would slow crude oil decomposition and prolong toxicity, relative to field conditions. Methane emissions from Louisiana freshwater marshes account for approximately 30% of total C emissions [17], far exceeding the 11% of C emissions as methane from these microcosms. The faster emission of methane under field conditions probably resulted from the greater volume of anoxic soils underlying field soils. Despite these differences, key elements of wetland soil organic matter mineralization were maintained in the microcosms throughout the incubations. The maintenance of an oxic-to-anoxic gradient was probably the most important characteristic for this experiment, because the interface between oxic and anoxic zones restricts nutrients from reaching overlying oxic waters [43]. Methane emissions combined with a high surface Eh throughout the experiment indicate that the vertical zonation of microbial processes, characteristic of wetland soils and resultant oxic/anoxic interface, was maintained in the microcosms. Similar carbon emission rates at the beginning and end of the 6-month incubations suggest that the microcosms were self-sustaining and somewhat homeostatic, two theoretical characteristics of representative microcosms [57].

Gross metabolic activity was not inhibited by crude oils or additives. But lack of inhibition at the community level does not necessarily mean an absence of toxicity. It is also possible that community-level activity was maintained by tolerant species that could consume substrates other than those they obtain in undisturbed soil. Numerous observations that crude oil reduced microbial numbers or diversity in sea water and terrestrial soils [2, 3, 6, 34, 60], invertebrate abundance [7], and diversity of emergent plant communities [39] suggest that lack of toxicity was unlikely. It therefore appears that activity was maintained by tolerant species that could metabolize substrates normally used by sensitive species. This is consistent with the perception that interspecific competitive exclusion regulates community structure of active soil archaea and bacteria.

Gross metabolic activity was not only maintained, but was also temporarily accelerated, by some treatments. The additional C emissions contained 1.4 to 3.5 times more carbon than was added as crude oil; thus, soil organic matter was the source of much of the additional C. There are two likely reasons for the temporary, accelerated activity. Crude oils may have contained nutrients that limit metabolic activity, and these nutrients gradually became incorporated into refractory substances and thereby less available over time, as has been observed in wetland nutrient enrichment studies [59]. Alternatively, crude oils temporarily reduced bacterivore populations, which increased heterotrophic bacteria populations and activity, as has been observed in water column studies [37]. If toxicity to bacterivores stimulated activity, then all treatments receiving crude oil should have been stimulated. However, the fact that Louisiana crude alone did not stimulate activity suggests that the stimulatory effect resulted from removal of nutrient limitation rather than removal of grazing. If macronutrients stimulated activity, then fertilization with N and P should have stimulated activity. However, the fact that fertilization without oil did not stimulate activity suggests that microbial metabolism was limited by micronutrients contained in crude oil rather than by N or P. The fact that fertilizer plus crude most accelerated activity further suggests that once the limiting micronutrients were available, then N and P further stimulated metabolism. However, studies designed to determine the relative importance of grazing, macronutrients, and micronutrients on limiting soil microbial activity are needed before these suggestions are accepted as explanations. Regardless, crude oil generally increased soil organic matter mineralization rates and the resultant release of nutrients rather than slowing the processes as I anticipated. The increase in nutrient remineralization rates was not quantified, but Parton et al. [54], in their successful model of C, N, P, and S dynamics in terrestrial grasslands, assumed 30–80% of carbon released during soil organic matter decomposition

contained N and P that were remineralized. Although the availability of P and other mineral nutrients probably increases linearly with soil organic matter decomposition rates, N availability is complicated by denitrification, which can increase with organic matter consumption [10]. Regardless, acceleration of soil organic matter remineralization, and the ensuing release of nutrients, might explain stimulatory effects of petroleum hydrocarbons sometimes observed on emergent vegetation [38, 39].

Although experimental data can be used to measure changes in microbial activity or plant growth, judging the effects of these impacts on wetland ecosystem integrity is beyond the scope of this study [78]. However, it should be noted that accelerated soil organic matter remineralization and nutrient remineralization can have negative consequences. For instance, increased nutrient availability is causing undesirable changes in the Florida Everglades [8, 16]. Increased soil organic matter decomposition could reduce marsh vertical growth, and, thus, the rate of global sea level rise that coastal marshes can survive [48–50]. Most people would probably agree that the most desirable consequence of oil spills would be no effect on microbial activity.

It cannot be assumed that all concentrations of crude oil would stimulate gross microbial activity. This experiment used an application rate of crude oil (75% surface coverage) that, while common in experiments, is likely exceeded in some portions of most oil spills affecting wetlands. The oil:substrate ratio (1:150) was greater than that used by Foght et al. [29] (1:1,000) in their study of freshwater dispersants on lake sediments. The oil:dispersant and oil:cleaner ratios (1:5) were greater than that used by Foght et al. [29] (1:10). The oil:surface area was approximately 3.8 L/m^2, which is similar to low levels (4 L/m^2) used by Lin and Mendelssohn [39]. Complete coverage of water with 4 mm of oil results from applying 4 L/m^2; higher rates simply increase the thickness of the oil layer. Unfortunately, there are no previous studies of the effects of crude oil on microbial activity in wetland soils. Nonetheless, stimulation of microbial metabolism by crude oil is consistent with some previous work from other habitats. Previous researchers noted increases in soil respiration after organic matter [22, 35, 67] and hydrocarbon mixtures [38] were added. Also, Alexander and Schwartz [2] noted that South Louisiana crude and Kuwaiti crude oils stimulated glucose mineralization in water column studies. Nichols et al. [47] noted that natural gas increased microbial biomass in aerobic sandy soils. Creosote in subsurface sandy clay sediments increased biodegradatory activity of the microbiota [77], increased microbial biomass, and decreased nutritional stress [63]. Chronic high levels (33.3 g C m^{-2} day^{-1}) of a 10-hydrocarbon cocktail inhibited microbial activity and plant growth in salt marsh soil, but chronic low levels (3.33 g C m^{-2} day^{-1}) stimulated them [38].

The most likely reason for an increase in the proportion of C emitted as CH_4 is a increase in methanogens relative to methanotrophs. Methanotrophs are likely more sensitive to changes in the volume of oxic soil because they require CH_4 produced by methanogens, as well as atmospheric O_2. The decline in the portion of C emitted as CH_4 between the beginning and end of the incubations combined with the similarity in total C emissions at the beginning and end suggests that energy flow in the microcosms became more efficient during the 6-month incubations. This increase in efficiency could have resulted from less flooding, from an increase in methanotrophs, or from development of fungal hyphae (which facilitate aerobic respiration in otherwise anoxic wetland soils [40, 52]). The greater importance of CH_4-C in *P. hemitomon* soils than in *S. lancifolia* soils suggests that greater activity in *P. hemitomon* soil shrank the volume of oxic soil. This, in turn, limited the opportunity for methanotrophs to intercept CH_4 before it escaped the soil. This explanation is supported by the lower Eh observed in *P. hemitomon* soil, which suggests a steeper oxic-to-anoxic gradient in those soils. The percentage of C emitted as CH_4 was not affected by the additives, but Arabian crude added to *S. lancifolia* soil elevated CH_4 emissions to levels similar to that in *P. hemitomon* soil.

Soil Eh was temporarily lowered by the crude oils and chemical additives. High Eh in bayou sediments containing refined hydrocarbons and heavy metals has been attributed to heavy metal stress inhibiting microbial metabolism [11]. Wilsey et al. [76] observed that fertilizers lowered Eh in salt marsh soil. Oil dramatically lowers soil Eh when it prevents oxygen from entering the water [18]. These, however, are the first data relating the effects of less-than-total oil coverage on soil Eh. The lower Eh observed in this study in response to crude oil and fertilizer is consistent with faster metabolism by anaerobic soil microbes, and increased demand for electron acceptors. The effects of lowered soil Eh on emergent marsh vegetation cannot be predicted from this study, but should depend on how much the surface oxidized layer is reduced. Although Eh was not measured deeper in the microcosms, the surface Eh measurements combined with the CH_4 emissions clearly indicate that the gradient from oxic surface to anoxic subsurface was steepened by adding crude oils.

In summary, there was no indication of decreased metabolic activity in response to crude oils, nor of reduced nutrient mineralization rates. Instead, Louisiana plus fertilizer, and all Arabian treatments, temporarily accelerated gross metabolic activity. It appears that the cleaner may be considered to prevent plant mortality or wildlife fouling without fear that the mixture of oil and cleaner will permanently alter soil microbial activity. Based on the similarity in responses of microbial activities to crude oil in *P. hemitomon* and *S. lancifolia*-dominated marshes, there is no reason to use different protection and clean-up strategies for these different marsh types. However, greater sensitivity to crude oil by *P. hemitomon* than *S. lancifolia* [39] suggests that higher priority be given to *P. hemitomon* when resources are limited.

Both crude oils temporarily lowered soil Eh. The effects that lowered soil Eh has on emergent marsh vegetation cannot be predicted, but will depend on how much the surface oxidized layer is reduced. The 50 mV decline in soil Eh might negate any effect that increased nutrient availability may have on plant growth. Studies using rooted vegetation are needed. It is also critical to note that this experiment used an application rate of crude oil (75% surface coverage) that, while common, is likely exceeded in some portions of most oil spills affecting wetlands.

Lastly, the fact that crude oils stimulated, rather than slowed, community level activity in this experiment does not contradict theoretical and experimental conclusions regarding relationships among biodiversity, ecosystem stability, and ecosystem production [45, 69]. Neither do these data contradict experimental conclusions that some components of crude oil are toxic. However, these results do indicate that crude oils also contain components that temporarily stimulate tolerant microbes. An interesting question is whether the tolerant species play relatively minor roles in undisturbed systems, as would be expected if community-level activity depended on species diversity, or if tolerant species dominate undisturbed systems, as might be expected if community-level activity depended on functional diversity [see 33, 70, 74].

Acknowledgments

This study was funded by the Louisiana Applied Oil Spill Research and Development Program. Mr. Gerry Canevari (G.P. Canevari and Associates) provided advice regarding treatment application. Exxon Research and Engineering Company provided the crude oils, the cleaner, and the dispersant. Mr. Roger Vincent (Miami Corporation) allowed access to Miami Corporation property to collect soil, and provided the services of their biologist, Mr. Billy DeLany, and an airboat. Mr. Mike Windham (Louisiana Department of Wildlife and Fisheries) suggested locations for soil collection and allowed cores and bulk soils to be collected from the Lake Salvador Wildlife Management Area. Dr. Don Davis (The Louisiana Applied Oil Spill Research and Development Program) tolerated much. The clarity of this manuscript benefited from three anonymous reviewers of an earlier version.

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