Application of Resource-Ratio Theory to Hydrocarbon Biodegradation

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Resource-ratio theory is one of the major logical frameworks used in ecology to predict how competition for growth-limiting resources influences biological diversity and function within a biological community. This paper presents experimental and other evidence that resource-ratio theory can also be applied to nutrient supplementation in hydrocarbon biodegradation. The theory makes two major predictions about nutrient supply and hydrocarbon biodegradation: (i) that changes in nitrogen (N) and phosphorus (P) supply ratio conditions will significantly alter biodegradation rates and that these changes in N:P ratio will cause significant shifts in microbial community composition and (ii) that changes in absolute nutrient supply levels, at constant supply ratios, will alter total hydrocarbon-degrader biomass, resulting in altered rates of hydrocarbon biodegradation. Here we analyze data from previous experiments studying the effects of N and P supply conditions on hexadecane and phenanthrene degradation in soil slurry reactors. Through the development of resource supply maps relating degradation rate and N and P supply conditions, it is shown that hydrocarbon degradation in slurry reactors is consistent with both major predictions of the theory. Further supporting evidence from previous studies on crude oil degradation in Arctic soils, hydrocarbon degradation in marine oil spills, and hydrocarbon composting in soils is also discussed in the context of resource-ratio theory. The paper suggests that more specific experimental studies are needed to verify further the utility of the theory and that, once more extensive data are available, the theory should provide a strong theoretical foundation for optimizing nutrient formulation and application strategies for hydrocarbon degradation in the future.

Introduction

Most organic pollutants can be transformed by natural microbial communities given sufficient time and appropriate ecological conditions. The practice of bioremediation has resulted from the use of such microbial populations to facilitate the degradation of such environmental contaminants. Although system heterogeneity and oxygen supply are centrally important factors that influence the outcome of practical bioremediation efforts in many physical settings, a limited supply of secondary nutrients can also limit rates of organic contaminant degradation (1–3). For example, supplies of the essential macronutrients nitrogen (N) and phosphorus (P) frequently limit microbial activity in many natural environments (3). One strategy for enhancing the success of bioremediation, therefore, is to stimulate microbial activity by the addition of one or both of these potentially growth-limiting nutrients (2, 4–6).

Although nutrient amendment strategies employed to enhance biodegradation have been reasonably successful (7), nutrient supplementation does not always achieve satisfactory results. In some instances, such as the recent study of Braddock et al. (4), both enhancement and inhibition of microbial activity have been observed following nutrient amendments. We believe that this limited success results in part from the fact that no theoretical framework for nutrient supplementation yet exists that allows us to reliably predict the best nutrient supply conditions for any given remediation effort.

In this paper, experimental evidence is presented that suggests that microbial responses to nutrient amendments in hydrocarbon-contaminated systems are consistent with the general predictions of resource-ratio theory (8–10). This ecological theory is one of the major logical frameworks used to predict how competition for growth-limiting resources can influence biological diversity and function within a biological community. During the past 15 years, this theory has been used to explain variations in the community structure and function of many environments, including species succession and nitrogen fixation by terrestrial vascular plants (11, 12); species succession and nitrogen fixation by phytoplankton in freshwater lakes (8, 13); the nutrient metabolism and community dynamics of heterotrophic microbial populations (10); and even the microbial ecology of periodontal disease in humans (14). We propose that this important ecological theory can similarly provide insights into how nutrient amendments can influence both the rate and the extent of organic pollutant biodegradation. We also believe that resource-ratio theory can help explain why less than optimal contaminant removal sometimes occurs under apparently high soil nutrient supply conditions and can also help to provide general guidance for improving nutrient supplementation strategies in future bioremediation efforts.

This paper will first review some basic principles of resource-ratio theory and its general predictions for bioremediation and then will reanalyze the results of several recent experimental studies in the light of this theory (15–17). These previous studies detailed the effects of inorganic nitrogen and phosphorus supplements on the rates of hexadecane and phenanthrene biodegradation in soil slurry microcosms. Finally, it will be shown how resource-ratio theory can help to explain observed variations in biodegradation success, particularly in scenarios where initial soil nutrient supply conditions appeared to have been acceptable.

Theoretical Background

Resource-ratio theory predicts that the biomass, community structure, and function of a biological assemblage will vary predictably with changes in the rates of supply and the ratios of potentially growth-limiting resources provided to the system (8). Because of phenotypic tradeoffs, it frequently has been found that organisms that may be competitively
superior for one growth-limiting resource are not competitively superior for other resources. Such phenotypic tradeoffs have been noted in both macro- and microorganisms (10). If different species (or strains) of microorganisms resident in a hydrocarbon-contaminated soil have different requirements for growth-limiting inorganic nutrients such as N or P, resource-ratio theory predicts that shifts in microbial dominance should occur across N and P supply gradients. If these different species then also exhibit varying abilities to metabolize the contaminant hydrocarbons, such shifts in microbial species composition should in turn cause changes in the rate of contaminant biodegradation. Maximum rates of biodegradation thus should occur at resource supply ratios favoring dominance by species that are most effective at hydrocarbon metabolism. The discussion below presents several key elements of resource-ratio theory that are most relevant to bioremediation. A much more detailed development of the theory can be found elsewhere (8–10, 18).

As can be seen in Figure 1a, the crossing of the isoclines for two essential resources creates regions of competitive exclusion and dominance at steady state. For example, resource-ratio theory predicts that none of the three organisms A–C will be competitively successful at resource supply points located in region NG. In theory, their growth will be restricted because insufficient resources are available to support a positive net growth rate for any of the three species under the given resource conditions ($\mu > -\mu$).

However, for resource supply points in the regions labeled...
A resource-ratio theory predicts that species A and B will be limited by resource 1 and that species A will ultimately outcompete species B by virtue of its ability to grow more efficiently on resource 1. Similarly, for resource supply points in regions B and C, either species B or species C, respectively, will be competitively dominant (8). In contrast, in the two regions labeled A + B and B + C, two different microbial species can coexist because one of the two species is theoretically limited by resource 2, and the other is limited by resource 1. Within this region, the relative biomass of both species will be very sensitive to changes in the N:P supply ratio. For example, for resource supply points placed along the resource gradients shown in Figure 1a, the relative biomass of species B will decline linearly from 100% to 0% with changes in the N:P supply ratio on either side of the two borders for region B (Figure 1a). If species B is capable of metabolizing a hydrocarbon contaminant that the other two species cannot degrade, then parallel changes in the rate of contaminant biodegradation should also occur (see Figure 1b).

Although the ZNGIs for only three different microbes are illustrated in Figure 1a, the general theory can also be applied to an indefinite number of potential microbial competitors such as may co-occur in natural soils (8, 12). A resource-ratio theory predicts that species with high hydrocarbon-degrading capabilities, then a corresponding enhancement of the rate of contaminant hydrocarbon biodegradation would theoretically occur. Stimulation of the biodegradation rate with increasing nutrient supply rates would occur up to the point (i) where the supplied nutrients become toxic or growth inhibiting, (ii) where other nutrient resources become growth limiting, or (iii) where the contaminant concentration itself is depleted sufficiently for it to become the rate-limiting resource.

Experimental Tests of the Theory

By extension, resource-ratio theory makes two important testable, implicit predictions about the responses of hydrocarbon-degrading microbial communities to changes in the supplies of growth-limiting nutrients. First, it predicts that changes in resource supply ratios should lead to changes in the rate of hydrocarbon biodegradation and that these changes should be accompanied by detectable shifts in microbial species composition. Second, if the resource supply ratio is held constant, resource-ratio theory predicts that changes in absolute nutrient supply levels should alter total hydrocarbon-degrader biomass and, thus, should cause changes in the rate of hydrocarbon biodegradation at that resource supply ratio. In the section below, empirical data from three recent experimental studies are reanalyzed to test these two theoretical predictions (15–17). In these studies, hexadecane and phenanthrene (two major components of petroleum spills) were used as model hydrocarbon contaminants.

Summary of Experimental Methods.

The experiments reexamined here used laboratory microcosms to assess the effects of differing inorganic N or P supply conditions on the rate of degradation of hexadecane or phenanthrene in soil slurries (15–17). Detailed descriptions of procedures employed have been provided previously; therefore, only a brief summary of the methods will be provided here (17).

These experiments used agitated, aqueous soil slurries consisting of a mineral salts medium, a model contaminant hydrocarbon (either hexadecane or phenanthrene), and 34 different N and P supply combinations provided as reagent-grade NaN_3 and/or K_2HPO_4. Each microcosm consisted of a 165-mL Teflon septum-sealed amber serum vial containing 60 mL of modified NSM medium (22); 3.0 g of air-dried soil; 203 mg of either hexadecane or phenanthrene (57.5 mg of C/g of soil); and between 0 and 8.4 mg of N/g of soil and 0.0–0.68 mg of P/g of soil of added N or P, respectively. The widely varying N and P supply combinations were selected to represent a broad range of limiting-nutrient conditions and also to create a matrix of both N- and P-limited growth conditions in the different microcosms. This simple soil slurry-based experimental design was used to provide consistent mass transfer conditions for each nutrient treatment and to reduce the effects of soil heterogeneity on the observed biodegradation responses. Similar methods have been used successfully in experiments elsewhere to assess the general microbial activity in soils (19, 20) and in previous hydrocarbon biodegradation experiments (4, 21).

Determination of CO_2 Production Rates and Yields, and O_2 Utilization Rates.

Hydrocarbon biodegradation was determined by monitoring both the production of CO_2 and the utilization of O_2 over time in the microcosms (with two duplicates per treatment). CO_2 accumulation was measured in the microcosm headspace, and O_2 uptake rates were measured respirimetrically on subsamples collected from the respective microcosms. Microcosm headspace CO_2 levels were analyzed using a Carle 311 analytical gas chromatograph equipped with a Poropack Q 80/100 column; O_2 utilization rates were measured using a YSI 5300 biological oxygen monitor. Details of both procedures have been reported.
elsewhere (17). All experiments used sterile, no-hydrocarbon, and no-nutrient controls to confirm that the CO$_2$ and O$_2$ responses were primarily biogenic.

Statistical curve-fitting techniques were used to estimate CO$_2$ production rates and final yields from time series data collected for each microcosm. The first-order model described by Simkins and Alexander (23) was found to provide the best estimates of CO$_2$ production yields from these data (17). As defined here, CO$_2$ production yield is the maximum mass of CO$_2$ produced under a given nutrient supply treatment (initial contaminant hydrocarbon levels were constant in all experiments). CO$_2$ yield estimates from the hexadecane experiments are provided in tabular form in Graham et al. (17). CO$_2$ production and O$_2$ utilization were highly correlated in our systems [e.g., the respiration quotient for the hexadecane-degrading slurries was 0.83 mg of CO$_2$/mg of O$_2$; $r^2 = 0.84$, $p < 0.05$ (17)]; therefore, CO$_2$ yield was used for comparisons among the experimental treatments.

**Soil Chemistry, Chemical Methods, and Definitions of Soil N and P Bioavailability.** The soil used in these experiments was collected from an oil field transfer site near Lawrence, KS (for soil handling details see ref 17). The soil was a sandy silt with gravel (ASTM D2487 Uniform Soil Classification System); it had a mean pH of 7.8, and it had mean soil C, soil N, and soil P levels of 13.1 mg of C/g of soil (range 11.5–14.1 mg of C/g), 0.96 mg of N/g of soil (range 0.64–1.3 mg of N/g), and 0.09 mg of P/g of soil (range 0.034–0.12 mg of P/g) by dry weight, respectively. The mean concentrations of four major soil N and soil P subfractions were as follows: inorganic soil N, 0.034 mg of N/g of soil; organic soil N, 0.93 mg of N/g of soil; Olsen soil P, 0.064 mg of P/g of soil; and non-Olsen soil P, 0.026 mg of P/g of soil. Olsen-P is the sodium bicarbonate-extractable fraction of total soil P and theoretically consists of both inorganic soil P and soluble organic soil P (24).

The analytical procedures used to estimate soil C, soil N, and soil P are detailed in Graham et al. (17). Total soil C and total soil N were both determined using a Carlo-Erba C/N analyzer. Analyses of the subfractions of soil N were performed using spectrophotometric methods and ion chromatography on samples extracted using 1.0 M KCl solution (25, 26). All soil P analyses were performed using standard spectrophotometric methods following acid-persulfate digestion (26, 27).

Because the predictions of resource-ratio theory are based upon estimates of available nutrient supplies, consistent definitions of bioavailable soil N and soil P supplies were essential to the analyses presented below. The soil nutrient measurements summarized above thus were used to discriminate between microbially bioavailable and nonbioavailable soil N or P. Graham et al. (17) were able to define the effective supply levels of available N or P in the soil examined here as (i) bioavailable N, measured inorganic soil N plus externally supplied N; and (ii) bioavailable P, measured Olsen soil P plus externally supplied P. These definitions of available N or P were used for all resource-ratio plots presented here.

**Enumeration and Identification of Microbial Populations.** In addition to CO$_2$ yields, microbial enrichment methods were used during the phenanthrene degradation experiments to detect any shifts in microbial community structure that accompanied observed changes in the rates of phenanthrene biodegradation (16). These measurements were performed on subsamples collected from selected microcosms 14 days after the initiation of the experiments, at a time when the instantaneous microbial CO$_2$ production and O$_2$ utilization rates in the microcosms were at or near maximum values. The analyses were performed using serial dilution and plating on phenanthrene-coated, NSM noble agar plates using techniques similar to Kastner et al. (28) and Kiyohara et al. (29). Phenanthrene was initially dissolved in acetone (2 mg of phenanthrene/4 mL of acetone), and 1 mL of this substrate was then spread onto each plate. After drying, the plates were inoculated with 100 mL of serially diluted slurry, spread, and incubated at 23°C in the dark for 2 weeks. Enumeration of zone-forming colonies was conducted using light microscopy under 100× magnification.

Microorganism identification was performed using a variety of traditional taxonomic measures (see Table 1) (16). Preliminary screening for Gram-negative and Gram-positive bacteria was performed using the Fisher Gram Staining Kit GSO-100 (Fisher Scientific). Cell morphology was determined under 1250× magnification using phase-contrast microscopy. Catalase reactions were assessed using colony reactivity with 3% hydrogen peroxide solution, and oxidase activity was assessed using differentiation disks (Difco). The dominant microorganisms enriched under each treatment were subsequently plated on Biolog Bugum agar, and tentative species identification was made using Biolog II techniques.

**Biodegradation Response Mapping.** The graphical software Surfer was used to develop response contour maps that related measured CO$_2$ production yields to the supply levels of bioavailable N and P under the various treatments (30). In general, these maps were developed by interpolating the data using a kriging method and then smoothing the data using a kriging gridding method. A linear variogram model was used in the kriging analyses for estimating all contours. The various response surfaces presented here were created using procedures similar to those used in computerized topographic contour map development.

**Results**

**Contaminant Biodegradation.** Our previous experiments examined the quantitative relationship among external nutrient supply levels, soil nutrient bioavailability, and hexadecane and phenanthrene biodegradation in soil slurries (15–17). It was found that changes in both the nutrient supply rate and the N:P supply ratio resulted in significant changes in biodegradation rate and CO$_2$ yield for these two contaminants. These studies did not, however, assess the degree to which the experimental responses agreed with the predictions of resource-ratio theory. Empirical tests of these predictions are presented below. In general, when CO$_2$ yields from the hexadecane and phenanthrene experiments were

<table>
<thead>
<tr>
<th>microorganism designation$^a$</th>
<th>Gram stain</th>
<th>oxidase</th>
<th>morphology</th>
<th>colony color</th>
<th>tentative identification$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>(−)</td>
<td>(+)</td>
<td>rod</td>
<td>pink</td>
<td>nonenteric</td>
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<tr>
<td>H</td>
<td>(−)</td>
<td>(+)</td>
<td>cocci</td>
<td>red</td>
<td>Micrococcus</td>
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<tr>
<td>I</td>
<td>(−)</td>
<td>(+)</td>
<td>rod</td>
<td>white</td>
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<tr>
<td>J</td>
<td>(+)</td>
<td>(+)</td>
<td>rod</td>
<td>white</td>
<td>Actinomycetales</td>
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<tr>
<td>K</td>
<td>(+)</td>
<td>(+)</td>
<td>coryneform</td>
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<td>Corynebacterium</td>
</tr>
</tbody>
</table>

$^a$ Alphanumeric nomenclature selected for reference only. $^b$ Tentative identification based on Biolog II and other conventional taxonomic analyses.
plotted as a function of the bioavailable N and P supply levels (see Figures 3–5), the patterns of CO₂ production were found to be consistent with predictions described in Figures 1 and 2. Figures 1 and 2 predict (i) that degradation activity should increase as the absolute supply level of N and P increase (at constant N:P ratios) and (ii) that local maxima and minima of degradation activity should be apparent at different N and P supply points and/or ratios. Figures 3–5 clearly show that these predictions are fulfilled in the hexadecane and phenanthrene degradation results.

For example, Figures 3 and 4 illustrate the strong effects of N and P additions on hexadecane degradation. Figure 3 provides the raw data used to perform the mapping, and Figure 4 provides the resulting response contour map constructed using Surfer. Figure 4 shows that comparatively low CO₂ yields were observed at N:P ratios greater than ca. 11.1 and less than ca. 5.1 (by mass) for hexadecane degradation, whereas maximal levels of degradation occurred at an intermediate N:P supply ratio of about 7:1. This unimodal response to the N:P supply ratio gradient strongly resembles the theoretical pattern predicted in Figure 1, panels a and b, and suggests that a N:P supply ratio of about 7:1 selected for a microbial subpopulation was most effective at hexadecane metabolism. These data do not necessarily imply that hexadecane-degrading subpopulations were absent at other supply ratios. However, the data do suggest that the microbial populations present at other supply ratios were less effective at hexadecane degradation than those dominant at a ratio of 7:1.

The data presented in Figures 3 and 4 also support the general theoretical predictions illustrated in Figures 1 and 2. Figures 3 and 4 show a strong decline in CO₂ yield as the N or P supply rates were reduced to zero; this diminishing response is consistent with the predicted effects of reduced resource supply rates (Figure 2). Similarly, as the total nutrient supply rate was increased at a constant N:P supply ratio (for example, at an N:P ratio of 7:1), CO₂ yield increased progressively to a maximum value at ca. 3.0 mg of N/g of soil and 0.45 mg of P/g of soil. This CO₂ yield maximum likely corresponds to the point at which the N or P supply levels ceased to be growth-limiting or possibly where another unmeasured essential mineral was sufficiently depleted to become growth-limiting. Graham et al. (17) suggested previously that this effect likely resulted from shifts from N- and/or P-limiting conditions to C-limiting growth conditions in the microcosms. On practical level, Figure 4 also suggests that hexadecane biodegradation in the soil examined here was much more sensitive to changes in the supply of N than to changes in the P supply. Such a differential response to N and P additions has been frequently observed in other hydrocarbon remediation scenarios (1–3, 5, 7).

Figure 5 illustrates the response contour map for phenanthrene degradation. Interestingly, Figures 4 and 5 indicate that phenanthrene and hexadecane biodegradation patterns are quite different under varying N and P supply conditions. For example, hexadecane biodegradation exhibited a single optimum at an N:P supply ratio of 7:1. In contrast, phenanthrene biodegradation appeared to have two N:P supply ratio optima, one closer to 5:1 and another at about 20:1. Phenanthrene biodegradation yield was reduced somewhat at supply ratios between these two optima.

This bimodal response pattern for phenanthrene is qualitatively and quantitatively consistent with the scenario illustrated in Figure 1, panels c and d. Figure 1, panels c and d, suggest that multiple degradation maxima are possible if there are metabolically different but also effective microbial populations that can consume a particular substrate. On a hypothetical level, our data suggest that at least two potentially different effective phenanthrene-degrading subpopulations can be selected, one at low and the other at higher N:P supply ratios. Alternately, our data also suggest that less effective phenanthrene-degrading populations can be selected under intermediate N:P supply conditions. Both of these conditions can be explained in the context of resource-ratio theory.

**Microbial Species Shifts.** If the biodegradation responses shown in Figures 4 and 5 were caused by resource competition and shifts in the microbial community composition, parallel analyses of community structure should show changes in the associated distribution of microbial species. Preliminary microbiological evidence from the phenanthrene experiments indeed supports this hypothesis.

Enrichment culture measurements made during the phenanthrene experiments revealed marked shifts in species composition in response to different N and P supply conditions (see Tables 1 and 2). Table 1 lists major characteristics of the five predominant microorganisms consistently observed in these microcosms. Although only tentative microbial identification could be made, distinctly different microorganisms were found to dominate under differing N and P supply conditions. Table 2 shows that microorganism H was dominant when N and P supply levels were not balanced; K was predominant when P supply levels were high, but N levels were low; and that microorganism I prevailed when external supplements of both N and P were provided and when N and P supply levels were more balanced for microbial activity (i.e., N:P ratio was 5:1 by mass). Although the data are not extensive, the data generally suggest that changes in the N and/or P supply conditions result in shifts in microbial community dominance which in turn appears to translate into different rates of hydrocarbon degradation (see Table 2). These shifts in microbial community structure are consistent with the predictions of resource-ratio theory.

**Discussion**

**Corroborating Evidence for the Applicability of Resource-Ratio Theory.** Although the use of external nutrients to

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**FIGURE 3.** Hexadecane biodegradation CO₂ production yields as a function of the bioavailable N and P supply levels (17). Inset numbers refer to the CO₂ yields, in milligram of CO₂-C per microcosm, at each respective N and P supply point. Available N is defined as supplied N plus inorganic N and available P is defined as supplied P plus Olsen soil P.
enhance bioremediation rates has increased dramatically over the past few years (2, 4, 6, 17, 31–33), nutrient formulations and amendment strategies have remained largely empirical. The empirical nature of these strategies results from recognition of the physical and chemical complexity of most contaminated sites and, on a more basic level, from a general lack of understanding of how microbial communities are affected by changes in nutrient supply conditions. We believe that the development of a formal theoretical framework for predicting microbial responses to supplied nutrients, such as resource-ratio theory, would greatly enhance our ability to explain and predict the effects of nutrients on microbial performance during bioremediation.

The experimental data presented in Figures 3–5 demonstrated clearly that both the N:P supply ratio and the absolute N and P supply levels strongly impact the rates of hexadecane and phenanthrene biodegradation in soil slurries. Moreover, the observed patterns of response are consistent with the general predictions made by resource-ratio theory. Several important questions must still be asked, however, about the actual utility of resource-ratio theory in the practice of bioremediation.

The first important question to be asked is, how broadly applicable is the theory? In principle, if the theory is truly robust and of real practical value, response patterns similar to those described here should be evident in other studies that have been performed using different environmental...
settings with different hydrocarbon contaminants. The results of such studies do, in fact, provide independent support for the theory. For example, in the presence of a single microbial species, the degradation response to nutrient enrichment should produce a relatively smooth response surface map that reflects the species’ unique metabolic requirements for growth-limiting resources. Such a response example can be seen in the recent work of Ishihara et al. (33). These authors examined the response of an oil-degrading strain (SM8) grown in artificial seawater microcosms that were experimentally contaminated with crude oil. They found that the rate of crude oil biodegradation responded strongly to manipulations of N and P and presented a three-dimensional plot of modeled O$_2$ utilization rates as a function of N and P supply levels. This plot exhibited a smooth, continuous response surface with little vertical relief and no evidence of multiple optima. The plot also showed that low O$_2$ utilization rates occurred at low N or P supply levels, and progressively increasing O$_2$ utilization rates resulted as N and P levels were increased. Resource-ratio theory would predict these simple degradation responses where no microbial species turnover occurred and when N or P were important limiting nutrients.

In contrast, the response of multiple microbial species should result in a more complex contour map that reflects both changes in total microbial density and shifts in microbial community structure. For example, a unimodal response to varying N:P supply ratios was strongly evident in the classic early study of Atlas and Bartha (31), with maximum rates of oil biodegradation in seawater occurring at N:P supply ratios...
TABLE 2. Dominant Phenanthrene-Degrading Species Enriched under Five Different Nutrient Supply Conditions

<table>
<thead>
<tr>
<th>nutrient supply</th>
<th>species enriched from microcosm (% of total population)</th>
<th>CO2 yield^d</th>
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<tr>
<td></td>
<td>G</td>
<td>H</td>
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<tr>
<td>initial soil conditions</td>
<td>3</td>
<td>31</td>
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<td>&lt;1</td>
<td>39</td>
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<tr>
<td>external P added only</td>
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<tr>
<td>external N added only</td>
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<tr>
<td>high N and P supply levels</td>
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^a Bioavailable N and P supply levels per gram of soil in slurry. Concentrations: 1. native soil N and soil P only; N = 0.03 mg/g and P = 0.10 mg/g. 2. external P added only; N = 0.03 mg/g and P = 0.39 mg/g. 3. external N added only; N = 1.83 mg/g and P = 0.10 mg/g. 4. low N and P supply levels; N = 0.39 mg/g and P = 0.15 mg/g. 5. high N and P supply levels; N = 1.83 mg/g and P = 0.39 mg/g. ^b The proportion of each phenanthrene-degrading species as a percentage of the total enumerated phenanthrene-degrading population, based upon colony morphology and plate counts. Colonies that formed phenanthrene clearing zones were considered phenanthrene-degrading populations. Samples collected after 14 days of exposure to phenanthrene when CO2 production rates were high. Total plate counts ranged from 3.2 x 10^8 colonies/g of soil to 7.9 x 10^8 colonies/g of soil depending upon the treatment. ^c Estimated CO2 production yields based first-order model [see Graham et al. for details (17)]. Units in mg of CO2-C produced per microcosm. The initial phenanthrene level was 171.5 mg of C per microcosm.

between 2.1 and 20.1 by moles; this example is analogous to that observed experimentally for hexadecane in Figure 4. Loya Nakan (32) similarly observed that N and P supplements decreased the doubling times of indigenous bacteria by approximately 40% and enhanced the rate of biodegradation of Prudhoe Bay crude oil in cold-climate soils. He noted that while N appeared to be the most stimulatory nutrient, additions of P with N resulted in statistically enhanced rates of carbon mineralization even in relatively fertile soils. Furthermore, he also demonstrated that the relative population densities of different microbial guilds shifted dramatically as nutrient supply conditions were altered.

Similarly, Braddock et al. (4) observed that while N was the major limiting nutrient at a hydrocarbon-contaminated site near Barrow, AK, population sizes and activities of soil microorganisms were maximally enhanced by additions of both N and P. They also found that microbial distribution patterns (the relative population densities of total heterotrophs, diesel-degraders, and gasoline-degraders) and patterns of hydrocarbon degradation (hexadecane vs pyrene loss rates) differed significantly under different N and P supply conditions. Resource-ratio theory, once again, can provide a plausible explanation for these changes in microbial activity and community structure.

Practical Implications of the Theory. The second important question that must be asked about the theory is, can it be practically applied in real-world scenarios? All of the above evidence suggest that hydrocarbon degradation is consistent with the general predictions of resource-ratio theory. However, is the theory of practical use? Can the theory be used to predict the effects of differing nutrient supply conditions a priori, particularly under the very wide range of environmental conditions that can exist in the field? In theory, if physiological traits and mortality rates of different microbial species are known, then resource-ratio theory could theoretically be used to make explicit predictions about the responses of mixed microbial communities to manipulations in their nutrient supplies (8–10). As our knowledge of environmental microbiology increases, we will likely have increasing access to these important data. In the interim, however, we suggest that resource-ratio theory can be used in the same way that it has been applied to help guide water resource management practices (13).

Resource-ratio theory predicts that the success of a given hydrocarbon bioremediation effort should depend on the supply rates and ratios of potentially growth-limiting nutrients to the system and also upon the identities of the microbial strains or species that are initially present and available for selection. Our experimental data suggest that, when paired with laboratory- or field-based response contour maps, the theory can be used to help predict the nutrient supply rates and ratios that should be the most appropriate to achieve optimal biodegradation activity for a given contaminant. For example, Figures 4 and 5 have illustrated how response maps can provide practically useful information about the effects of varying N or P supplies on hexadecane- and phenanthrene-biodegrading microorganisms from the soils studied here. Although the hydrocarbon concentrations used in our experiments were high as compared to soil carbon levels and only one soil type was employed, the results clearly show that the theory is applicable in our case (which is a relatively common case in nature) and that the further testing of theory under other degradation scenarios is warranted.

Braddock et al. (4) have recently stressed that a good understanding of nutrient effects at a specific site is essential for successful bioremediation, and we believe that a carefully designed series of microcosm bioassays can be used to generate site-specific nutrient response maps. While these response maps cannot reasonably be expected to be an absolutely perfect predictor of future performance, we suggest that this mapping procedure nonetheless can provide a cost-effective method to predict the responses of a given biodegradation system to varying nutrient additions for a particular soil or marine scenario. We also believe that these procedures can provide guidance for the types and amounts of nutrients that should be supplied.

Moreover, these practical tools can also be used to examine the effects of other important local environmental factors that influence the response of the indigenous biodegrading microbial populations to nutrient supplies and supply ratios. For example, low-moisture soils receiving high supply rates of inorganic N have sometimes been found to exhibit comparatively low rates of biodegradation relative to soils containing higher moisture contents. Braddock et al. (4) suggested that, even at the modest rates of nutrient fertilization used in their study of JP-5 contaminated soils, the low soil water content may have resulted in aqueous concentrations of nutrients that were high enough to be toxic. Such responses should be generally predictable using the microcosm-based bioassay approach. In another example, a response surface map from a recent study in which both the N supply level and the soil slurry water content were experimentally varied revealed that intermediate water levels produced optimum crude oil biodegradation rates in slurry reactors (34); lower and higher water levels produced lower rates of degradation. In general, such response surface maps are easily interpreted and can potentially be used to explore the interactions among nutrient supplies, moisture levels, and other key environmental factors such as soil temperature or oxygen levels.
Resource-ratio theory predicts that maximum rates of biodegradation should be observed at resource supply ratios that favor dominance by microbial species, strains, or guilds that are most effective in metabolizing the target contaminants. Although this basic conclusion appears to be somewhat obvious, resource-ratio theory goes beyond this simple prediction. We argue that resource-ratio theory provides plausible mechanistic explanations for three key questions central to the practice of bioremediation: (i) Which growth-limiting resource(s) most strongly influence rates of biodegradation in a given environment? (ii) Why do multiple optimal resource supply conditions for biodegradation exist? (iii) Why do certain resource supply combinations negatively affect biodegradation effectiveness?

It has been recognized since the seminal studies of Atlas and Bartha that N:P supply ratios can be an important determinant of biodegradation success, and resource-ratio theoretical questions can only be provided through empirical explanation for these observations. Moreover, nutrient amendments are often made using a fixed N:P addition ratio, and resource-ratio theory suggests that it is the effective supply rates and ratios that determine the actual responses of contaminant-degrading microbes to externally supplied nutrients. The bioremediation literature historically has most commonly emphasized N as the most common limiting nutrient in terrestrial hydrocarbon biodegradation (e.g., refs 1, 3, 5, and 7). However, it has been suggested that P availability may be critical in some settings, especially where soil moisture levels might be low (4, 6, 17, 32). We suggest that the procedures outlined here can be used to help guide practical decisions about the nutrients that are most likely to be growth-limiting and help define the subsequent nutrient application rates. As discussed by Graham et al. (17), estimates of bioavailable soil nutrient supplies can be incorporated into these considerations in order to maximize the effectiveness of external nutrient supplementation and to optimize the quantity (and costs) of nutrients provided to a biodegradation system.

It is also evident from the multiple optima observed in Figure 5 that the microflora present at a given site may be composed of two or more metabolically different subpopulations, with different N and/or P supply needs, that are effective at contaminant biodegradation. In such cases, the response surface mapping procedures outlined above could be used to predict the nutrient application conditions that should be avoided (such as those that would produce local biodegradation rate minima) and to help identify the nutrient amendment rates and ratios that would be needed to optimize subsequent rates of biodegradation.

We suggest that more detailed answers to these and other practical questions can only be provided through experimental studies aimed specifically at assessing the resource supply responses that occur under different biodegradation conditions. The structure and function of soil microbial communities can be strongly influenced by both deterministic and nondeterministic factors. In this paper, we have focused on testing the predictions made by equilibrium resource-ratio theory, using laboratory-scale microcosms. In natural soil systems, which may be subjected to significant environmental variability, the actual composition of soil microbial communities may track the equilibrium states predicted by resource-ratio theory only imperfectly. For example, temperature, predation, excretion of growth-inhibiting substances by members of the microbial assemblage, and temporal variance in resource supply rates all can modify the outcome of resource competition. However, the potential effects of these environmental factors can be evaluated directly using careful experimental methods (9, 18). It is also clear from the ecological literature (e.g., ref 35) that stochastic factors, such as the timing of species introductions, can alter the dynamics of multispecies assemblages such as the soil microbial communities discussed here. It thus will be very important to assess the degree to which real-world environmental conditions modify the outcomes predicted by the theory, and we urge that carefully designed field-scale tests also be performed in order to test the broader applicability of resource-ratio theory to in situ hydrocarbon biodegradation.

It is hoped that this paper will encourage further microcosm-based experiments similar to those described here in order to assess the effects of resource supply ratios across a wider variety of contaminants and biodegradation scenarios. If the applicability of resource-ratio theory is broadly confirmed, we suggest that this theory could be used to predict the detailed biodegradation responses of different classes or types of organic contaminants to nutrient additions. We can envision a future situation in which the amounts and ratios of nutrients added at a given bioremediation site could be tailored successfully both to the soil chemistry of the site and to the identity of the organic contaminant(s) that are present.

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