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Assimilation of Oil-Derived Carbon and Remedial Nitrogen Applications by Intertidal Food Chains on a Contaminated Beach in Prince William Sound, Alaska

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ABSTRACT

Beaches in Prince William Sound, Alaska were heavily oiled by the Exxon Valdez spill in March 1989. Fertilizer supplements designed to stimulate oil degradation by indigenous bacteria were applied to beaches in the rocky intertidal zones. We used stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes to monitor trophic interactions following these treatments. Representative macroalgae and consumer organisms were sampled during the summers of 1989 and 1990. Microcosm experiments were conducted to trace bacterial assimilation of fertilizer nitrogen and to compare relative uptake of oil and indigenous substrates during 1990.

Fertilizer nitrogen was the dominant source of bacterial nitrogen in the microcosm experiments. Bacteria preferentially assimilated indigenous substrates and used oil as a substrate only after more labile substrates were depleted. Following depletion of indigenous substrates, nitrogen supplements appeared to enhance assimilation of carbon from oil. The $\delta^{13}C$ and $\delta^{15}N$ values of bacteria growing on oil and fertilizer nitrogen were distinct in the rocky intertidal environment, providing a tracer to examine the link between bacterial production and higher trophic levels. No evidence, however, was found for a transfer of carbon or nitrogen assimilated by bacteria to higher trophic levels. © *1997 Elsevier Science Ltd. All rights reserved*

INTRODUCTION

Various methods to facilitate environmental clean-up and recovery in the rocky intertidal zone were used following the *Exxon Valdez* oil spill in Prince William Sound, Alaska. One approach used fertilizers to stimulate indigenous bacterial activity toward degradation of oil (see Pritchard & Costa, 1991). Part of this bioremediation program included monitoring

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environmental effects resulting from the fertilization treatment. We used stable carbon and nitrogen isotope ratios at the natural abundance to determine whether oil-derived carbon and fertilizer nitrogen were assimilated by bacterial communities within the rocky intertidal food web and subsequently transferred up the food chain.

Defining major energy flows is critical in understanding the effects of oil pollution and bioremediation treatments on shallow water and intertidal ecosystems. With this information, trophic levels involved with cycling the contaminant material can be identified. Both stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes have been used extensively to study elemental cycling and food chain structure (Peterson & Fry, 1987). In addition, a few studies have used stable isotopes to examine the contribution of anthropogenic elements to aquatic food chains (Macko & Ostrom, 1994). For example, the assimilation of sewage wastes by benthic macrofauna and fish has been studied in coastal California waters using $\delta^{13}C$ and $\delta^{15}N$ analyses (Spies *et al.*, 1989, Rau *et al.*, 1981). Spies *et al.* (1989) estimated that 15–20% of the organic matter consumed by some species of fish originated from sewage. Using an alternate approach, Tan and Walton (1975) measured the $\delta^{13}C$ value of dissolved inorganic carbon in seawater to estimate the degree of eutrophication in various waters. They observed that dissolved inorganic carbon was isotopically lighter (^{12}C-enriched) in more eutrophic waters compared with control stations. These studies demonstrate the valuable use of stable isotopes to trace anthropogenic carbon and nitrogen sources into marine food chains.

While stable isotopes have been used to describe food chains and to trace the source and fate of organic matter in aquatic ecosystems for years, they have been used only recently to study the processing of organic matter by bacteria. New approaches have made it possible to assess the relative importance of salt marsh detritus, phytoplankton, riverine and anthropogenic sources of organic matter to bacterial production (Coffin *et al.*, 1989, 1990; Coffin & Cifuentes, 1993). Complementing these isotopic approaches for examining bacteria are analytical methods for measuring $\delta^{13}C$ values of dissolved organic matter (Peterson *et al.*, 1994) and $\delta^{15}N$ values of nitrate, ammonium and dissolved organic nitrogen (Velinsky *et al.*, 1989, Cifuentes *et al.*, unpublished data). With these methods, the sources and fate of carbon and nitrogen can be traced through the bacterial assemblage.

We report on the use of stable isotopes to monitor the bacterial assimilation of carbon in petroleum spilled from the *Exxon Valdez* and fertilizer nitrogen used to stimulate oil bioremediation in the rocky intertidal zone. In turn, we discuss the subsequent incorporation of these elements into higher trophic levels. Parallel microcosm experiments were used to simulate bioremediation conditions, providing a model system to study bacterial assimilation of oil-derived carbon and fertilizer nitrogen and to link microbial processes to higher trophic levels in the intertidal food chain.

METHODS

Laboratory experiments

A microcosm experiment was conducted with four treatments of oiled gravel from Disk Island: (1) fertilizer addition; (2) fertilizer and seagrass detritus addition; (3) seagrass detritus addition; and (4) no additions (control). Well-sorted oiled gravel from Disk Island was placed in 20-liter Nalgene tanks to the 2.5 liter mark. CUSTOMBLEN granular

fertilizer containing 28% N and 8% P was added at concentrations of 1 kg m^{-2} of oiled gravel to fertilized treatments. This amendment was equivalent to augmentations used on field plots sampled from Disk Island. Seagrass additions (20 g) consisted of debris from the high tide line at a Disk Island beach which was dried at 50°C and crushed to a fine powder.

Tidal flushing in the microcosms was simulated by exchanging water daily. Each day, filtered (0.2 μm) fresh Prince William Sound water was added to the 3.5 liter mark of the microcosm through a spigot at the base of the container. After 12 h, the water was drained through the spigot and the gravel was exposed to air for the next 12 h. This cycle of inundation and exposure continued for 13 days. The experiment was performed at ambient laboratory temperatures (about 15°C).

The water recovered from the microcosms was initially filtered through a 1 μm Nuclepore cartridge filter. An aliquot of this filtrate was retained for measurements of bacterial abundance (Hobbie *et al.*, 1977). A portion of the 1 μm filtered sample was then filtered through a GF/F filter (baked 450°C over night). The filter was immediately frozen and stored at –20°C prior to isotopic analysis. At t = 24, 96 and 192 h, the remaining portion of the filtrate was concentrated by tangential flow filtration and nucleic acids were extracted from the < 1.0 μm concentrates (Coffin *et al.*, 1990) for stable carbon isotope analysis.

Field sampling protocol

Samples of algae, consumer organisms, bacteria, particulate organic matter and interstitial water were taken from a beach on Disk Island in Prince William Sound, Alaska during the summer of 1990 (Fig. 1); the species that were examined are described below. This area was heavily contaminated with oil during the March 1989 oil spill from the tanker *Exxon Valdez*. Sampling was closely coordinated with researchers who were studying bioremediation treatments and samples were collected from a 3 × 3 m test plot that was treated with 1 kg m^{-2} CUSTOMBLEN granular fertilizer (Pritchard & Costa, 1991, Pritchard *et al.*, 1992).

Helicopters or seaplanes were used to transport researchers to field sites. We attempted to sample on a weekly schedule; however, actual dates depended on weather and aircraft schedules. Organism samples taken from the beach were stored in coolers, transported back to the laboratory and frozen within 4 h. Water samples were either transported to support vessels near the beaches for processing or flown back to the laboratory in Valdez. The time between sampling and processing of water samples varied between 1 and 4 h.

The organisms chosen for this study inhabit a large area of the intertidal zone. By taking advantage of the unique niches colonized by different organisms, a large area of the intertidal zone was monitored isotopically. Representative green, brown and red macroalgal species included *Urospora* sp., *Fucus disticus* and *Odonthalia* sp., respectively. Consumer organisms sampled included those from various feeding strategies such as the suspension feeder, *Balanus glandula* (barnacle), surface feeders, *Littorina sitlcuma* (periwinkle) and *Tacetara persona* (limpet), and the higher trophic level predators, *Nucella emegginata* (whelk) and *Anoplarcus purpurceus* (eel blenny).

The above algae and heterotrophic organisms were immediately placed in plastic bags and frozen using dry ice. Larger organisms were rinsed in ambient water prior to freezing. Prior to preparation for stable isotope analysis, samples were stored in a laboratory freezer

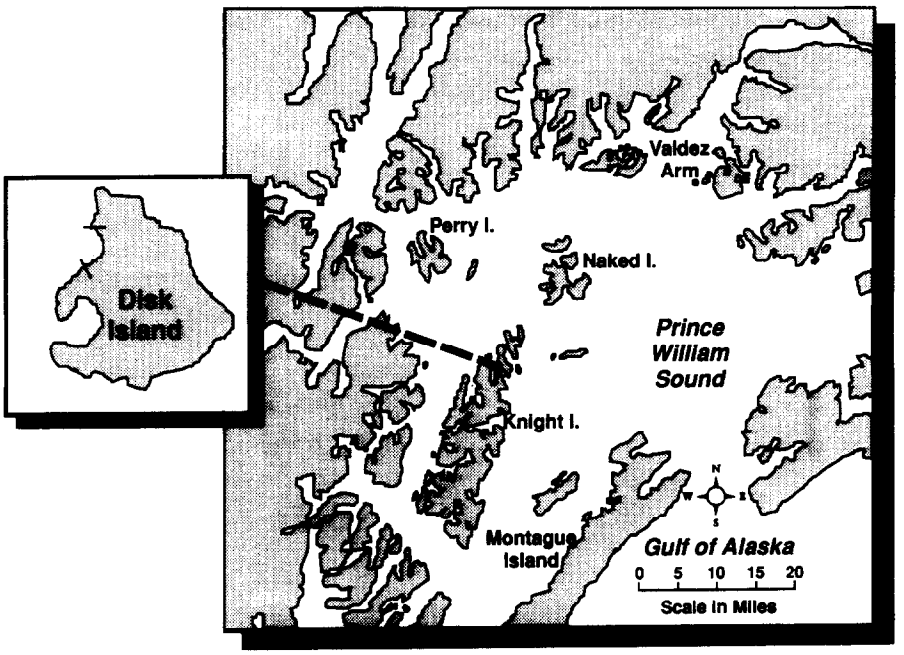

Fig. 1. Sample site on Disk Island in Prince William Sound, Alaska. Area between hash marks on the island represent the study locations.

at –20°C. In the laboratory, samples were thawed, rinsed copiously with distilled water and freeze-dried. Four replicate organisms were then ground together using a mortar and pestle and stored in vials in a desiccator before isotopic analysis.

In the field study, extractions of nucleic acids (Coffin *et al.*, 1990) followed by stable carbon isotope analysis were used to examine *in situ* sources of bacterial substrate (described above). Bioassay incubation experiments (Coffin *et al.*, 1989) were performed to assess the potential for bacteria to use oil as a substrate source. The bioassay incubation involves filtering seawater samples through a 0.2 μm filter, inoculating the water samples with a 1% addition of 1.0 μm-filtered water and incubating the water sample in the dark until the bacterial biomass is sufficient to obtain a stable carbon or nitrogen isotope value (usually 48 h),

In the coastal waters, suspended particulate organic matter was used as a proxy for stable carbon and nitrogen isotope values in phytoplankton, as the phytoplankton may not be separated from other components of the particulate organic matter. Particulate organic matter was also collected from interstitial and beach waters by filtration on a 47 mm glass fiber filter (Whatman GF/F, ashed at 450°C for 4 h). The filters were frozen immediately and stored at –20°C. Prior to isotopic analysis, filters were placed in a desiccator with HCl fumes to remove carbonates, followed by drying at 60°C for 24 h and grinding.

Finally, 1–2 liters of the filtrates from the collection of particulate organic matter were placed in Nalgene bottles, frozen on dry ice and transported to the laboratory for storage (–20°C). We analyzed for the stable nitrogen isotope values of the NO_3^- and NH_4^+ in these samples.

Stable carbon and nitrogen isotope analysis

All samples were analyzed isotopically using a modified Dumas combustion that converts carbon and nitrogen to CO_2 and N_2 gas for mass spectral analysis (Macko, 1981; Cifuentes *et al.*, 1989). Preparation and analyses of dissolved ammonium and nitrate are described elsewhere (Velinsky *et al.*, 1989; Cifuentes *et al.*, 1989). Carbon isotope ratios were analyzed on a Finnigan MAT 251 IRMS and nitrogen isotope ratios were analyzed on a Nuclide 3-60-RMS.

Stable carbon and nitrogen isotope ratios are reported according to the standard formula:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where δX is either $\delta^{13}C$ or $\delta^{15}N$ and R is either $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard for carbon was PeeDee Belemnite (PDB). The standard for nitrogen was ultrapure tank nitrogen that was standardized against atmospheric N_2. The analytical precision of the measurement for $\delta^{13}C$ of organisms and particulate organic matter was $\pm 0.2‰$. For $\delta^{15}N$, the analytical precision was $\pm 0.3‰$ for organisms and particulate organic matter and $\pm 0.5‰$ for NO_3^- and NH_4^+. We estimate that the analytical precision for the bioassay and nucleic acid techniques is in the range of $\pm 1.0‰$ (Coffin *et al.*, 1989, 1990; unpublished data).

RESULTS AND DISCUSSION

Before tracing the fate of bacterial biomass in bioremediated field sites, we conducted a laboratory study to test whether bacteria growing on oil-derived carbon or fertilizer nitrogen possessed a unique isotopic ratio. In the microcosm experiment, we compared different carbon (seagrass and oil-derived carbon) and nitrogen (indigenous and fertilizer nitrogen) sources. Although inherently complex, this controlled laboratory investigation did not suffer from problems encountered with beach heterogeneity and sampling frequency at the Disk Island site.

Microcosm experiments

Seagrass was added to two microcosms in order to compare the isotopic ratio of bacterial biomass grown on isotopically different substrates, oil and seagrass, which had $\delta^{13}C$ of 30.1‰ and −16.8‰, respectively. Nutrients were added to enhance biodegradation (Pritchard & Costa, 1991; Pritchard *et al.*, 1992).

Bacterial abundance in the untreated or unamended microcosm (control) effluent started at approximately 20×10^9 cell liter^{-1} and decreased throughout the course of the experiment, reaching a minimum of approximately 5×10^9 cell liter^{-1} (data not shown). Decreases through time indicated that daily flushing was effectively removing bacteria from the microcosms. At all times, treatments with added seagrass carbon and nutrients did not have significantly greater bacterial biomass compared to the control. Thus, neither enhancement with seagrass carbon nor fertilizer nitrogen stimulated bacterial production sufficiently to overcome the influence of the simulated tidal flushing.

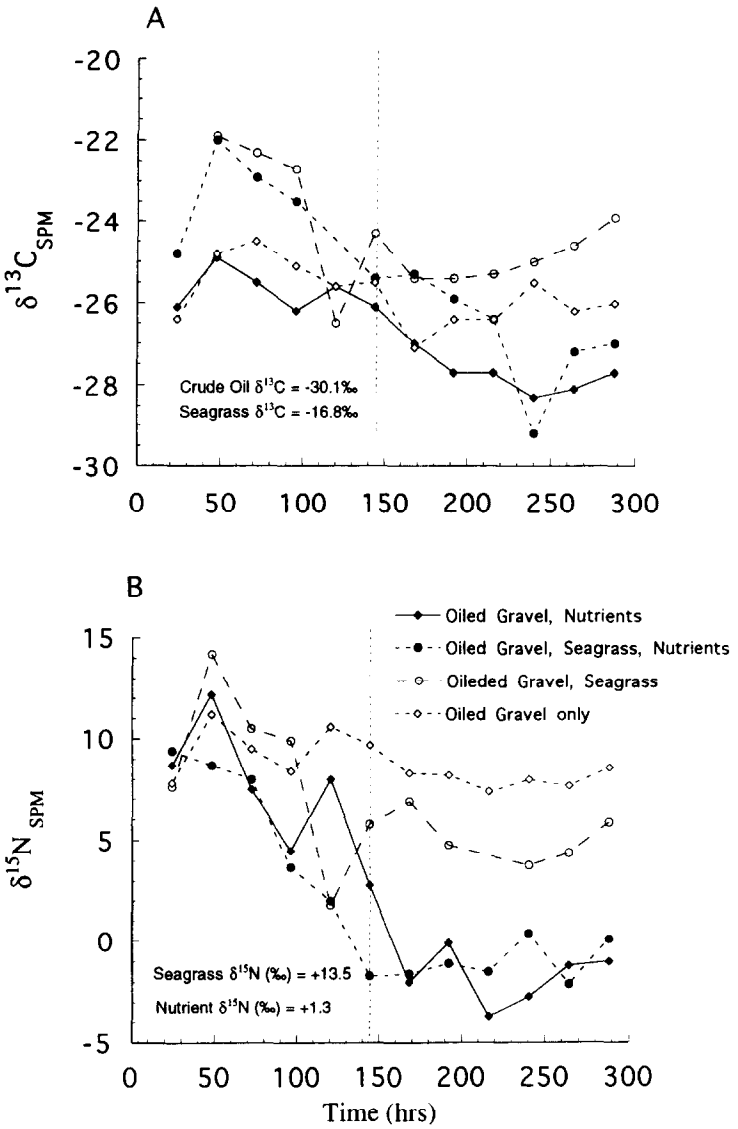

Fig. 2. Stable carbon (A) and nitrogen (B) isotope values of < 1.0 μm particles versus time in the microcosm experiments. Data to the right of the line shown at $t = 148$ were used to generate Fig. 3.

Definite isotopic variations were observed in the microcosm experiment both as a function of time and treatment. Associated with the apparent removal of bacteria over time, there was a trend of decreasing $\delta^{13}C$ and $\delta^{15}N$ (Figs 2(A) and (B)) in the < 1.0 μm particles ($P_{< 1\,\mu m}$) which included the bacteria. In the latter half of the experiment ($t = 144$ to 288 h), when bacterial abundance experienced little change, $P_{< 1\,\mu m}$ were significantly 13C-enriched (t-test, $P < 0.05$) in the microcosm amended with seagrass carbon compared to the control (Fig. 3). In contrast, addition of nutrients alone resulted in significant 13C-depletion in $P_{< 1\,\mu m}$ (t-test, $P < 0.05$). Enhancement with both nutrients and seagrass, however, produced no significant carbon isotopic change.

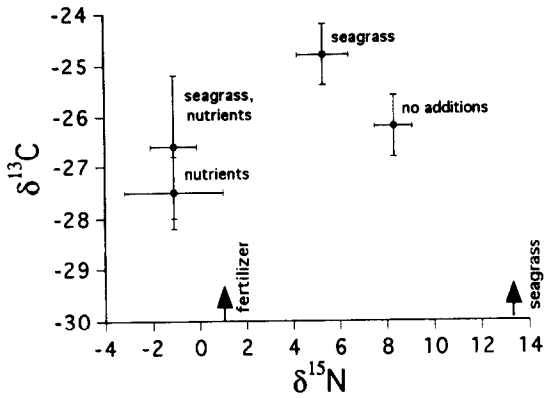

Fig. 3. Mean and standard deviation ($\pm 1\sigma$) of $\delta^{13}C$ and $\delta^{15}N$ in < 1.0 μm particles collected from $t = 148$ to 288 h in the microcosm experiments. Refer to Fig. 2. The $\delta^{15}N$ of fertilizer nitrogen and seagrass are also shown.

If $P_{< 1\,\mu m}$ included inorganic and detrital particles and adsorbed oil of similar size to bacteria, then isotopic variations with time and among treatments may have resulted from selective removal of $P_{< 1\,\mu m}$ components following daily flushing. While this could explain the ^{13}C-enrichment in the treatment with added seagrass detritus (greater retention of seagrass particles), it is not a convincing argument for the ^{13}C-depletion seen in the nutrient-only treatment. Simple addition of nutrients should not have influenced removal of < 1 μm particulate organic matter from the tanks.

It is arguable that isotopic changes observed in the microcosm may have resulted from algal production. Autotrophic growth could have only taken place on top of the gravel because daylight was excluded below the surface. No visual evidence of algae, however, was observed on the tank surfaces. Also, prefiltration, through 1.0 μm filters prior to concentration of bacterial-sized particles, would have eliminated most plankton recovered in the simulated tidal flushing (Coffin *et al.*, 1990). In turn, plankton growing in coastal seawater ($S = 33$ psu, $\delta^{13}C_{DIC} = 0$ to 1‰) would not typically reach values found in $P_{< 1\,\mu m}$ (−28 to −26‰) from fertilized tanks at the end of the experiment or in the nucleic acid extracts (see below). In fact, suspended particulate matter sampled off the Disk Island beach, probably representative of phytoplankton growing on dissolved inorganic carbon found in the water used in the experiment, only had $\delta^{13}C$ of −24 to −23‰.

We favor the explanation that the isotopic changes observed in $P_{< 1\,\mu m}$, although small, would be consistent with selective utilization of carbon sources. More positive $\delta^{13}C$ values indicated that seagrass (−16.8‰) was preferentially used as a carbon source for bacterial growth. Decreasing $\delta^{13}C$ would signal enhanced degradation of the ^{13}C-depleted oil (−30.1‰). This interpretation was also supported by the nucleic acid data described below.

The temporal trend of decreasing $\delta^{13}C$ found in all treatments (Fig. 2(A)) was more pronounced in the nucleic acid measurements (Fig. 4). At $t = 24$ h, nucleic acid extracts in microcosms that received seagrass additions had ^{13}C-enriched isotope ratios similar to that of seagrass detritus. Through time, the $\delta^{13}C$ of nucleic acid extracts became progressively more negative in all treatments. By $t = 196$ h, isotope ratios of nucleic acid extracts were uniform and ^{13}C-depleted compared with the $P_{< 1\,\mu m}$ (Fig. 3). Differences between

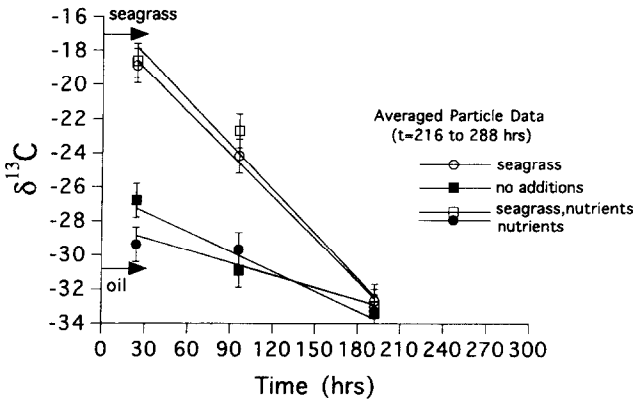

Fig. 4. Stable carbon isotope ratios of nucleic acid extracts versus time. The error bars indicate the typical precision of the measurements (Coffin *et al.*, 1990). Included are the mean $\delta^{13}C$ of < 1.0 μm particles taken after the last nucleic acid sample was collected ($t = 216$ to 288 h). The $\delta^{13}C$ of oil and seagrass are also shown for comparison.

nucleic acids and $P_{<1\,\mu m}$ can be understood if the latter fraction included organic matter other than bacteria (e.g. seagrass detritus, oil). Finally, following the discussion above, it is not likely that nucleic acid extracts were derived from autotrophic organisms.

The data in Fig. 4 suggested that early in the two seagrass treatments, this source of organic matter was preferentially used to build bacterial biomass. Based on the nucleic acid isotope data, we can approximate the relative contributions of petroleum and seagrass carbon to bacterial biomass. Assuming an isotopic discrimination of approximately +2.3‰ between bacteria and substrate (Coffin *et al.*, 1990) and that plant leachate is typically 2.0‰ more negative than the whole plant (e.g., *Spartina alterniflora*, Benner *et al.*, 1987), the $\delta^{13}C$ value of nucleic acids from bacteria growing solely on the seagrass mixture should be approximately –16.5‰, whereas that from growth on oil would be approximately –27.8‰. Therefore, at $t = 24$ h, isotope ratios of –18.6 to 7–18.9‰ in the seagrass-amended microcosms indicated that bacteria received about 80% of its carbon from seagrass and the other 20% from oil. Three days later ($t = 96$ h), the contribution from seagrass decreased to between 32 and 45%, with 55 to 68% deriving from the oil. Finally, after one week, the nucleic acid $\delta^{13}C$ became even more negative than the oil. We cannot explain this further depletion in ^{13}C at this time. A more thorough investigation would require $\delta^{13}C$ analysis of individual compounds of the oil using GC/IRMS.

The addition of fertilizer resulted in ^{15}N-depletion in both fertilizer-treated microcosms compared to the seagrass-added and control treatments (Fig. 2(B)). The seagrass (+13.5‰) used in the microcosms was ^{15}N-enriched in contrast to either NO_3^- (–2.4‰) or NH_4^+ (+0.3‰) in the CUSTOMBLEN fertilizer. Oil, of course, has minimal associated nitrogen so it is not surprising that the control had the most positive and unvarying $\delta^{15}N$ (Fig. 2(B)). With nitrogen limitation, remineralization becomes the dominant source and remineralization of nitrogen results in ^{15}N-enrichment (Hoch *et al.*, 1996). The influence of the fertilizer nitrogen only became pronounced after seven days ($t = 144$ to 288 h, Fig. 2(B)) when fertilizer appeared to be the major nitrogen source in the $P_{<1\,\mu m}$ fraction, even in the treatment with added seagrass (Fig. 3). It is possible that the slow uptake of fertilizer nitrogen resulted from bacterial preference for indigenous organic nitrogen

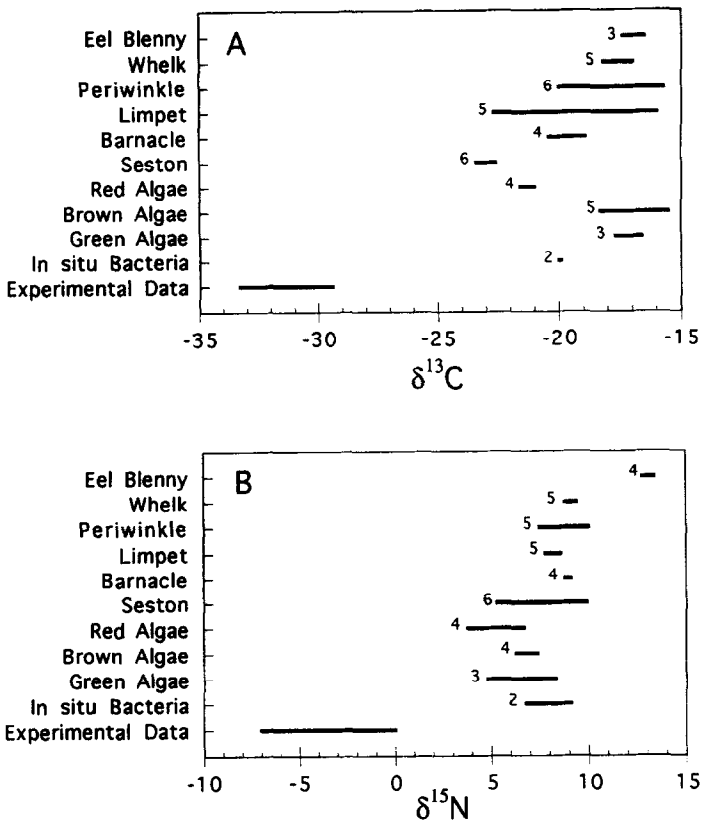

Fig. 5. Ranges of $\delta^{13}C$ (A) and $\delta^{15}N$ (B) for organisms on rocky intertidal zone from Disk Island in Prince William Sound, Alaska. The number of samples taken is included. For comparison, we added the range of bacterial isotope data measured in the microcosms and bioassay incubations.

sources. Earlier work comparing bacterial assimilation of inorganic and organic nitrogen sources suggests that organic nitrogen is preferred when available (Wheeler & Kirchman, 1986; Kirchman *et al.*, 1989). Alternatively, the lag in the isotopic shift may have been due to the slow-release nature of the inorganic fertilizer.

Field observations

Phytoplankton in Auke Bay and Fritz Cove in southeastern Alaska have $\delta^{13}C$ of -20.3 ± 0.9‰ and -20.3 ± 0.2‰, respectively (Goering *et al.*, 1990). Data for surface particulate matter reported by these authors, about -23 to -20‰, were only slightly more positive than values we measured about 100 m from the Disk Island beach (see seston data in Fig. 5(A)). Bacterial nucleic acids extracted from both interstitial and surface water also had $\delta^{13}C$ values (Table 1) within these ranges, consistent with phytoplankton being a major substrate source.

Bioassays using interstitial waters with and without CUSTOMBLEN fertilizer (Table 1), were more like those measured in the fertilized microcosm treatments (Fig. 3). An explanation for similar outcomes in all fertilized experiments is that more oil (-30.1‰) was

TABLE 1
Stable Carbon and Nitrogen Isotope Values of Bacteria from Disk Island During the Summer of 1990

Sample/Treatment	$\delta^{13}C_{na}$ (‰)	$\delta^{13}C_{ba}$ (‰)	$\delta^{15}N_{na}$ (‰)	$\delta^{15}N_{ba}$ (‰)
Disk Island cove water	−20.0(1)	−22.0(2)	6.8(1)	8.4(2)
Disk Island pore water	−19.9(1)	26.5(1)	9.1(1)	8.0(1)
Disk Island pore water and fertilizer nitrogen	n.a.	−27.4(1)	n.a.	−7.4(1)

Stable isotopes in bacteria were measured using bioassay (ba) incubation experiments or nucleic acids (na) as biomarkers of bacteria. See the text for an explanation of these data. Numbers in parentheses represent the number of replicates and the abbreviation n.a. indicates that the data is not available.

assimilated in the presence of added nitrogen. The reason for the ^{13}C-depletion in the unfertilized bioassay, however, was not clear, particularly in view of the more positive values found *in situ* (i.e. nucleic acids). Bioassays, however, only provide a measure of potential activity (Coffin *et al.*, 1989). Moreover, bioassays are closed systems that do not experience the continuous flushing occurring on the beaches. As implied by the bioassay data, it is possible that the potential for accumulating bacterial biomass, which assimilated oil, existed in these interstitial waters but did not occur in the beach environment (see below).

If the addition of fertilizer adds significant quantities of ammonium and nitrate, their isotopic signatures should be evident in interstitial waters. As stated earlier, these nitrogen species in CUSTOMBLEN fertilizer had $\delta^{15}N$ of +0.3 and −2.4‰, respectively, and were consistent with previous measurements of fertilizers (Macko & Ostrom, 1994). Showing the influence of the fertilizer application, the fertilized plot was ^{15}N-depleted −1.4‰. In contrast, ammonium in the control beach plot had a $\delta^{15}N$ of +8.8‰. This comparison between beaches was paralleled in the nitrate data. Its value in the treated plot was −2.3‰ compared to +5.6‰ in the control plot. Thus, the isotopic signal of fertilizer nitrogen was seen in interstitial waters of treated beaches.

The distinct $\delta^{15}N$ value found in ammonium from fertilized beaches was not observed in nucleic acids from interstitial waters or in the corresponding bioassay incubations (Table 1). Furthermore, the average for the unamended field samples (nucleic acid extracts and bioassays; $+8.1 \pm 1.0$‰, $n = 5$) was similar to that found in the untreated microcosm ($+8.3 \pm 0.8$) and the ammonium (+8.8‰). The addition of CUSTOMBLEN fertilizer to a bioassay with interstitial water, however, resulted in a significantly ^{15}N-depleted value, 7.4‰ (Table 1). This value was considerably more negative than any nitrogen sources found on the beaches, including fertilizer, and more negative than the fertilized microcosm treatments (-1.1 ± 2.1‰). There is, however, an isotope effect (−16‰) when bacteria assimilate ammonium at high concentrations (Hoch *et al.*, 1992). Although we did not measure ammonium concentrations, it is most likely that elevated ammonium concentrations existed in the bioassay and not the microcosm or field, which were both influenced by flushing. Thus, isotope fractionation probably occurred in the fertilized bioassay resulting in additional ^{15}N-depletion. Finally, the isotope effect for nitrate assimilation by bacteria is not known but bacteria typically do not use nitrate when dissolved organic nitrogen or ammonium are present (Keil & Kirchman, 1991; Hoch & Kirchman, 1995).

The rocky intertidal food chain

Stable carbon and nitrogen isotopes are commonly used to establish food chains (Peterson & Fry, 1987). Because fractionation of stable carbon isotopes is small between trophic levels (Rau *et al.*, 1983), direct correspondence of $\delta^{13}C$ values can be used to determine food source(s) for higher trophic levels. Also, consumers are generally enriched in nitrogen by about 3‰ compared to their food source (Minagawa & Wada, 1984). Based on the carbon (Fig. 5(A)) and nitrogen (Fig. 5(B)) isotope data, we described two trophic levels at the Disk Island site with algae at the base of the food chain and eel blennys at the highest trophic level sampled. The carbon isotope values spanned by consumer organisms (Fig. 5(A)) was similar to that reported by Dunton *et al.* (1989) in the southern Bering Sea and by Goering *et al.* (1990) in southeastern Alaska. The large range of values, however, suggested they fed on a mixture of several sources. This conclusion was also reached by Rau *et al.* (1991) when describing the trophic structure of invertebrates in the Weddell Sea.

Recent reports concluded that nutrient additions stimulated bacterial degradation of the petroleum on beaches (Pritchard & Costa, 1991; Pritchard *et al.*, 1992). At issue is the fate of the bacterial biomass that degraded the contaminant. If significant accumulation of bacteria, relying primarily on oil carbon and fertilizer nitrogen, occurred, then the distinctive $\delta^{13}C$ and $\delta^{15}N$ values measured in microcosm and field bioassay experiments should have been observed in the field. Neither bacteria, phototrophs or higher trophic level organisms sampled *in situ* exhibited these isotope ratios (Figs 5(A) and (B)). This implies that if bioremediation was effective, then the energy in bacterial biomass formed from oil degradation was not transferred efficiently (see Azam *et al.*, 1983) to higher trophic levels. Alternatively, high tidal action may have resulted in transport of bacterial biomass from the beach to coastal waters.

Closer inspection of isotope data from Disk Island and other beaches indicated that the fertilizer nitrogen was assimilated by at least one species of algae (Fig. 6). Bacteria, however, did not appear to be a significant source of energy or nutrients to higher trophic

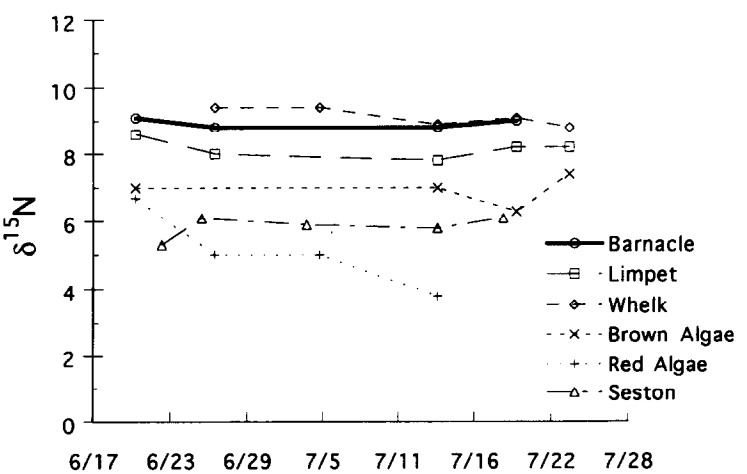

Fig. 6. Stable nitrogen isotope values of organisms sampled during nutrient addition experiments on Disk Island Beach.

levels in this environment. This trophic structure may have been particular to the Disk Island ecosystem. The physical dynamics of the rocky intertidal zone are dominated by high tidal energy and large volumes of water that are flushed through the porous gravel and cobble on the beaches. This probably limited significant accumulation of bacterial biomass even in the presence of high carbon and nitrogen availability. More stationary macroalgae, however, could remain on the fertilized beaches long enough to assimilate nitrogen and acquire its isotopic ratio. Thus, even if bacteria were assimilating oil-derived carbon and fertilizer nitrogen, they may not have reached concentrations necessary to support higher trophic levels. Finally, it is possible that undegraded, toxic components may have accumulated on the beaches and ultimately influenced the beach ecosystem, but our isotope data cannot address this issue.

CONCLUSIONS

1. Assimilation of oil and fertilizer nitrogen imparted a unique isotopic signature to the bacterial assemblage.
2. The distinct isotopic values of oil-derived carbon and fertilizer nitrogen were not found in bacteria, algae or higher trophic level organisms from Disk Island beaches.
3. If significant bacterial biomass was formed during oil degradation on Disk Island, it was not assimilated by organisms at higher trophic levels.

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