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Location and DGGE methodology can influence interpretation of field experimental studies on the response to hydrocarbons by Antarctic benthic microbial community

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Abstract: A field experiment investigating the effect of oil contamination on benthic microbial communities was conducted near Casey Station, East Antarctica. Defaunated sediment was treated with a mixture of Special Antarctic Blend diesel and lubricating oil and deployed in three different bays for eleven weeks. A molecular fingerprinting technique, denaturing gradient gel electrophoresis (DGGE), was used to investigate the microbial community structure. The variation between replicate samples within treatment groups indicates that the benthic microbial populations are very diverse and evenly distributed. Comparisons to determine the significance of both deployment location and hydrocarbon treatment showed that the greatest effect was from a combination of location and treatment. Detailed analysis suggests that subtle differences may be obscured by variability introduced by PCR and gel stages in DGGE, undermining this experimental approach. It is concluded that both location and hydrocarbon contamination influenced the development of the microbial communities but that the effect of hydrocarbon treatment varied with location. This has important implications for the design of future experiments on the effect of hydrocarbons on benthic communities, especially if it is intended to generalize the conclusions drawn from site specific studies.

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Introduction

The response of microbial communities to hydrocarbon contamination of sediment affects other trophic levels in benthic ecosystems, as microbes remove hydrocarbons from the sediment and incorporate them into the food chain (Danovaro 2000). The addition of petroleum hydrocarbons to marine sediment can increase the number and activity of benthic microbes (e.g. Griffith *et al.* 1981, 1982). However, many investigations have relied on culture-dependent techniques in laboratory and microcosm experiments (Prince 1993) with limited relevance to field situations. Recent advances in molecular microbial techniques for characterizing the composition of bacterial communities, such as denaturing gradient gel electrophoresis (DGGE), used in combination with well designed manipulative field experiments (such as randomised block designs) hold the promise of major advances in understanding the microbial processes involved in bioremediation (Head & Swannell 1999).

The few published accounts of manipulative field experiments using DGGE to look at the response of microbial population structure to hydrocarbons provide inconclusive and sometimes contradictory results. Macnaughton *et al.* (1999) observed changes in microbial community structure during bioremediation of an experimental (randomized block design) oil spill using DGGE and phospholipid analyses. Swannell *et al.* (1999), in a randomized block experiment, report that bacterial populations, analysed using DGGE, in untreated control plots and plots treated with fertilizer alone remained stable, while changes occurred in plots amended with oil and oil with fertilizer. Roling *et al.* (2004) used DGGE in randomised block experiment to compare bacterial community structure in plots treated with oil alone, oil plus liquid fertilizer, oil plus slow release fertilizer and a control. They found that the community structure in the plots treated with oil alone and with oil plus liquid fertilizer did not differ from the control, but there were significant differences between the plots treated with oil and slow release fertilizer. However, they found these differences varied with time and among different blocks and even within plots. Because of this variability they conclude that it may not be sufficient to sample a single location when the bacterial community structure of oil-contaminated shore lines is studied.

Some laboratory and microcosm experiments using similar techniques provide findings which are superficially more conclusive. However, the level of replication in these experiments is not comparable with that of the randomised block field experiments described above, thus limiting the scope for generalisations from these studies. Whiteley & Bailey (2000) using DGGE identified highly specialized but low diversity microbial communities in each of the different

stages of an industrial phenol bioremediation system. However, with only one system and without repeat sampling they have no indication of how representative the communities in each of the stages were. Roling *et al*. (2002) found very different microbial communities were selected in all of a series of beach sediment microcosms treated with oil and different levels of nutrients. Similarities between DGGE profiles of replicate samples from single microcosms were high, but similarities between samples from replicate microcosms subjected to similar nutrient additions were not significantly higher than those between microcosms subjected to different nutrient amendments.

A consistent theme amongst all these bioremediation experiments using DGGE is the highly variable nature of the results which may be due to the DGGE technique itself. DGGE has several advantages over traditional culture-based methods such as the ability to detect non-culturable microbes, but a single band on a gel is not necessarily from a single species (Buchholz-Cleven *et al.* 1997), the intensity of the signal is not related to the abundance of particular species (Murray *et al.* 1996) and it is subject to PCR and gel biases (Suzuki & Giovannoni 1996, Ferrari & Hollibaugh 1999, Powell *et al.* 2003). In addition, sample heterogeneity has also been identified as contributing to the variability of DGGE banding patterns (Nichol *et al.* 2003). These sources of variability have not been accounted for in the experiments described and may contribute to the difficulty in interpreting many of the results.

In a previous study to identify regional differences in microbial community structure we optimized the use of DGGE by identifying sources of variability in the method and using an approach that minimised these effects (Powell *et al.* 2003). In this current study we wished to determine whether these techniques for controlling the variability introduced by DGGE would improve sensitivity of the procedure and allow us to detect more subtle changes in microbial populations in manipulative field experiments. We also wished to test whether the results of manipulative field experiments looking at the effects of oil on microbial community structure are influenced by location as suggested by Roling *et al.* (2004). In our previous work at Casey Station, Antarctica, in the summer of 1998–99 (Stark *et al.* 2003) we demonstrated a causal link between differences in biotic communities at control and contaminated locations with the presence of hydrocarbons/heavy-metals, using a sediment recruitment experiment. Sediment was defaunated, artificially contaminated and then deployed in three different locations (Brown, O'Brien and Sparkes bays) for eleven weeks. The mixture of Special Antarctic Blend (SAB) diesel and lubricant oil had a significant effect on the recruitment of diatoms and infauna (Cunningham *et al.* 2003, Stark *et al.* 2003). In addition, the location at which the sediment was deployed in was also important. Communities recruiting in O'Brien, Sparkes and Brown bays were all different from each other regardless of the treatment applied to the sediment. Analysis of the hydrocarbon concentrations in samples from each bay indicated that the SAB diesel hydrocarbons had degraded in all locations, but significantly more so in Brown Bay, which showed high levels of biodegradation (unpublished data). The short-chain *n*-alkanes were barely detectable and in comparison to the pre-deployment samples, the concentrations of the isoprenoids (which are more recalcitrant to biodegradation than alkanes) had decreased. We hypothesized that the microbial communities in Brown Bay, which has been contaminated for at least 20 years, have adapted to utilizing hydrocarbons as a carbon source and as a consequence, new sources of hydrocarbons, such as those supplied in the experiment, could be utilized more efficiently in Brown Bay than in other locations.

In this paper we report the effects of hydrocarbon treatment on benthic microbial population structure at three locations and discuss how DGGE reproducibility, including variation due to gel run and PCR round, can influence the ability to detect subtle changes in population structure.

Methods

Experimental design and sampling

The experimental design is described in detail in Stark *et al.* (2003). Briefly, sediment was collected from O'Brien Bay, a pristine site near Casey Station. To defaunate the sediment it was frozen to -20°C, thawed and sieved through a 500 µm screen. The sediment was allowed to settle and excess water was removed. This sediment was then split into treatments. Nothing further was done to the control treatment. A mixture made up of 50 ml of SAB and 25 ml of synthetic lubricant oil was added to 55 l of sediment to construct the hydrocarbon treatment. It was stirred in and an additional 15 l of seawater was added to the slurry. This was allowed to settle overnight before the excess water was removed. Pre-deployment samples were collected at this time.

The control and hydrocarbon-treated sediments were placed into plastic flowerpots (12 cm deep and 12 cm in diameter) that had 3 holes (8 cm x 8 cm) cut in the side and a 9 cm diameter hole in the bottom. The holes were covered by 300 µm mesh to retain the sediment but allow water and oxygen exchange in the pots. Three trays of each treatment, containing six pots of sediment, were deployed in Brown, Sparkes and O'Brien bays (Stark *et al*. 2003 for further details) for *c.* 11 weeks during the summer. At the end of this time the trays were retrieved by diver and sediment from two pots from each tray were frozen at -20°C for chemical analysis and DNA extraction. The frozen sediment was divided into surface (0–2 cm) and sub-surface samples (< 2 cm). Only surface samples were analysed in this study.

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Table I. Mean similarity coefficients (%) calculated between samples from the same treatment group.

	A: Same gel (same PCR round)		B: Same PCR round (different gel)		C: Different PCR (different gel run)	
Treatment group	*n*	mean (range)	*n*	mean (range)	*n*	mean (range)
Pre-deployment	2	89 (77–100)	4	71 (67–75)	8	55 (40–67)
O'Brien control	4	57 (22–90)	8	71 (50–86)	12	57 (40–67)
O'Brien hydrocarbon	11	56 (22–80)	28	52 (18–89)	17	44 (20–91)
Sparkes control	1	86	4	55 (40–67)	8	51 (40–67)
Sparkes hydrocarbon	10	53 (25–100)	22	56 (36–100)	18	53 (17–75)
Brown control	5	52 (40–67)	11	50 (29–67)	9	54 (33–75)
Brown hydrocarbon	12	73 (43–100)	30	62 (33–91)	30	56 (40–92)

Analysis of microbial community structure by DGGE

DNA was extracted from the frozen sediment samples and amplified by two rounds of PCR as described in Powell *et al.* (2003). Conditions for the denaturing gradient gel electrophoresis were similar to those described in Powell *et al.* (2003). The denaturing gel was poured (6% acrylamide, 30–65% denaturant) to *c.* 2 cm below the bottom of the wells. A stacking gel of 10% acrylamide (0% denaturant) was poured on top. The gels were pre-run at 80V for 30 min before the wells were flushed out and half the volume of the PCR product was loaded. Gels were run at 80 V for 16 hours at 60°C in 1 x TAE. After the first 15 min, the run was paused whilst the wells were washed out again. Standards were run on either side of the gel and the outside lanes were not used. For even heat distribution throughout the tank it was placed on a magnetic stirring plate.

Gels were stained in 1:1000 Sybergold (Molecular Probes) in the dark with gentle shaking for approximately twenty minutes. They were then washed once with deionised water and destained with deionised water for twenty minutes before viewing on a UV transilluminator.

Gels were photographed with a digital camera and viewed with the UTHSCSA ImageTool program. The best possible banding pattern was obtained by enhancing the contrast and grayscale of the images and in some cases applying a rolling disk background subtraction. This banding pattern was then transformed into a presence/absence matrix for statistical analysis by scoring each band as present (1) or absent (0). The standards were used to check for gradient consistency between gels and to assist in comparing the position of bands between gels.

Primer5 statistical program (Plymouth Marine Laboratories) was used to analyse the banding patterns. The presence/absence data for each sample from three DGGE runs was combined such that for each sample, every band had a score of 0, 1, 2 or 3. A similarity matrix was constructed using the Bray-Curtis measure and a presence/absence transformation of the data. Community patterns were examined using non-metric multidimensional scaling (MDS) ordinations and the ANOSIM procedure (one-way) was used to test for differences between groups (Clarke & Warwick 1994).

Powell *et al.* (2003) observed that differences between gels contributed significantly to differences in the banding patterns obtained from multiple analyses of the same sample. To overcome this effect, samples were analysed multiple times and the presence/absence data combined. This procedure was also carried out for the current set of samples. In addition, the variation in banding patterns due to gel variability, PCR run variability and sample heterogeneity within a treatment group were explored by looking at the similarity coefficients between pairs of samples. The Bray-Curtis similarity coefficient was calculated on the presence/absence data for each pair of samples where each of the pair were from a single sample, amplified in a single round of PCR and run on the same gel. The similarity coefficients were also calculated for the same sample amplified within the same round of PCR and run on two different gels to determine the effect of different gels and for the same sample amplified in different rounds of PCR to determine the effect of different PCR runs. The mean and range of the similarity coefficients between different samples in the same treatment group were determined (Table I).

To compare treatment groups whilst excluding PCR and gel-to-gel variability, only samples amplified in the same

Table II. ANOSIM values comparing similarities of control and hydrocarbon treatments between and within locations. Significant differences are in bold.

Comparison	R statistic	Significance level
a Between pre-deployment samples and post-deployment treatments		
O'Brien control	0.287	0.17
O'Brien hydrocarbon	0.238	0.17
Sparkes control	0.167	0.20
Sparkes hydrocarbon	**0.460**	**0.04**
Brown control	0.324	0.09
Brown hydrocarbon	**0.500**	**0.02**
b Between control treatments		
O'Brien, Brown	0.266	0.14
O'Brien, Sparkes	-0.083	0.57
Brown, Sparkes	-0.083	0.63
c Between hydrocarbon treatments		
O'Brien, Brown	0.043	0.29
O'Brien, Sparkes	**0.325**	**0.01**
Brown, Sparkes	**0.289**	**0.03**
d Within location: control versus hydrocarbon treatment		
O'Brien	**0.367**	**0.02**
Brown	-0.147	0.84
Sparkes	0.259	0.11

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Table III. Ranking of treatment groups from most to least similar based on mean similarities calculated between samples from the same round of PCR run on the same gel. The mean similarity is given in brackets.

Similar Similarity > 70%		Similarity 60–69%		Similarity 50–59%		Different Similarity < 50%	
Pre	(89)	Brown C–Brown H	(66)	O'Brien C	(57)	pre–Brown C	(48)
Sparkes C	(86)	Sparkes C–Brown C	(66)	O'Brien H	(56)	O'Brien H–Sparkes H	(47)
Brown H	(73)	O'Brien C–Sparkes C	(65)	O'Brien C–Brown C	(56)	pre–Sparkes C	(46)
		Sparkes C–Sparkes H	(60)	pre–O'Brien C	(55)	pre–Brown H	(46)
		Pre–O'Brien H	(60)	O'Brien H–Brown H	(53)	pre–Sparkes H	(41)
		Sparkes H	(53)				
		Brown C	(52)				
		O'Brien C–O'Brien H	(51)				
		Sparkes H–Brown H	(51)				

Pre = pre-deployment, C = control treatment, H = hydrocarbon treatment

round of PCR and run on the same gel were compared. The similarity coefficients for each group of comparisons (e.g. O'Brien control–O'Brien hydrocarbon) were collated over all the gels and the mean determined (Table III).

Results

DGGE reproducibility and variation due to gel and PCR variability

By comparing the similarity of the banding pattern from a single sample subject to either different gel runs or both different PCR and different gel runs, an estimate of the variability due to gel and PCR effects was obtained. For samples for which the same PCR round was run on different gels (n = 9), the mean similarity was 54% (range 44–86%) and for samples subject to both different PCR rounds and different gel runs (n = 32) the mean similarity was 57% (range 22–100%). The samples subject to both sources of possible variation had a wider range of similarities than those subject only to one source of variability.

A measure of sample heterogeneity within the same treatment group is shown in Table I. When the similarity

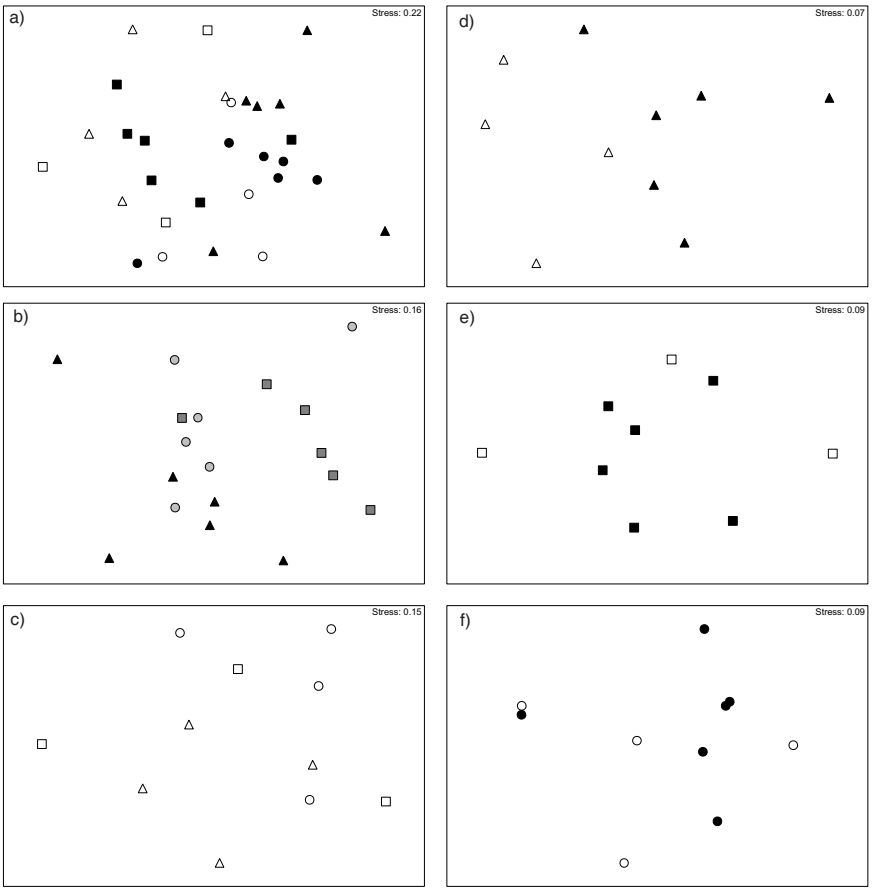

Fig. 1. MDS ordinations showing the relative similarities of microbial communities between the control samples (open symbols) and hydrocarbon treatment samples (closed symbols) deployed in Sparkes (■), Brown (●) and O'Brien (▲) bays: **a.** All samples, **b.** control treatments, **c.** hydrocarbon treatments, **d.** O'Brien Bay, **e.** Sparkes Bay, **f.** Brown Bay.

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coefficients are calculated on samples run in the same round of PCR and on the same gel (column A), the mean similarity is generally higher than when the coefficients are calculated on samples run either in the same round of PCR but on different gels (column B) or in different rounds of PCR and different gels (column C). In nearly every case, as the number of sources of potential variation increase, the mean similarity decreases. It was expected that replicate samples for each same treatment group run on the same gel would be very similar, but the mean similarity coefficients range from 52 to 89%. It is interesting to note that the homogenized pre-treatment samples had the highest similarity coefficients.

Effect of experimental procedure

The microbial communities in each treatment at the end of the experiment were compared to the original pre-deployment control sediment, the source of which was O'Brien Bay (Table IIa). None of the control treatments at any location were significantly different from the pre-deployment control sediment. There was no significant difference between the pre-deployment and hydrocarbon treatment at the source site of O'Brien Bay, and the only significant changes were seen in the hydrocarbon treatments at the non-source sites of Brown and Sparkes Bay (Table IIa).

Effect of location and hydrocarbon treatment

Figure 1a shows the relative similarity between all the samples on a single MDS ordination and it is difficult to see any clear overall pattern in response to treatment or location. The hydrocarbon and control samples were analysed as two separate MDS ordinations and some patterns relating to differences among locations can be seen, but only for the hydrocarbon treatment (Fig. 1b & c). There was no difference among locations for the control sediment (Table IIb, Fig. 1b), while for the hydrocarbon treatment Sparkes Bay was significantly different from O'Brien and Brown Bay (Table IIc, Fig. 1c). The hydrocarbon treatments in Brown and O'Brien bays are not significantly different from each other (Table IIc). The lack of differences among the control treatments may be because the variation within each location is as great as the variation between locations (Fig. 1b).

The mean similarity of different treatment groups was calculated by pair-wise comparison of samples that were run on the same gel. These paired comparisons were ranked by mean similarity and divided into four groups from most similar to most different (Table III). The most similar comparisons were samples from within the same treatment group whilst the most dissimilar were the comparisons of pre-deployment samples to those deployed in Sparkes and Brown bays. These rankings generally support the results of the ANOSIM tests (Table II) and both analyses detected differences between the pre-deployment samples and some of the post-deployment treatments. The implication is that both location and hydrocarbon treatment had an effect on the development of the sediment microbial communities.

The effect of the hydrocarbon treatment on the microbial communities was determined by comparing the control to the hydrocarbon treatment within each location (Table IId, Fig 1d, e, f). There was a significant difference between the two treatments in O'Brien Bay (Fig. 1d) but not in either Sparkes or Brown Bay (Table IId, Fig. 1e, f). The ranking of mean similarity coefficients (Table III) also suggests that the difference between the control and hydrocarbon treatments is most significant in O'Brien Bay.

Discussion

Methodological issues

The original purpose of the experiment reported here was to investigate the response of the benthic infauna to hydrocarbon and heavy metal contamination (Stark *et al.* 2003), and for this reason it was important to thoroughly defaunate the sediment first. This was achieved by freeze-thaw and sieving, which is not ideal for studying microbial populations as the freeze-thaw treatment would have killed most, but not all, of the sediment microbes and changed the original community structure. However, it has been assumed that all the treatment groups had the same remnant microbial population at the time of deployment. Thus at the end of the eleven weeks of *in situ* incubation the microbial communities were a combination of microbes that survived the freeze-thawing and those recruited from the surrounding environment, both of which may have been affected by the hydrocarbons. The differences between the pre-deployment samples and some post-deployment treatment groups show that the microbial communities did develop and change over the incubation period.

When analysing microbial communities by DGGE, there are three sources of variation that may influence the banding patterns in each treatment group. The first of these relates to variability within the DGGE method: from both the PCR and electrophoresis (gel) steps. The similarity of the banding pattern obtained from the sample pairs analysed either in different PCR rounds or on different gels was low (54 or 57%). This indicates that for each pair of banding patterns compared, only about half the bands were present in both and that with each round of PCR or DGGE bands from different species are detected. This suggests that not only is the diversity in these communities high (as described in Powell *et al.* 2003) but the populations are quite evenly distributed within a sample. If the communities were dominated by a few species the same bands would always be present. Thus combining several runs of the same sample will give a better estimate of the total community composition and diversity and reduce methodological biases.

The second source of variation in banding patterns relates to the high degree of variation in microbial communities

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between samples from the same treatment group. In this experiment, within treatment similarities ranged from 52 to 89% indicating a large amount of heterogeneity between samples. This range is probably a realistic estimate of small-scale sample variability in microbial communities and highlights the importance of adequate replication within treatments. Clegg *et al.* (2003) also observed that spatial variation obscured treatment effects in their study on the effects of grassland management practices. The effect of this within treatment variability is itself quite variable and appears to depend on the type of microbial community under investigation. In a study of oil bioremediation in a mudflat shoreline, Roling *et al*. (2004) found that generally within treatment differences were not significant, but at some sampling times they were. However, Calvo-Bado *et al*. (2003) measured a 96% similarity in the DGGE banding patterns produced from replicate DNA extractions from the same sand filter. In our study, the same trends are seen in both the ANOSIM values (Table II) and the mean similarity coefficients (Table III); thus differences can be detected in the DGGE banding patterns from our study despite these sources of variability.

The final and most important source of variation is that between treatment groups. To observe a treatment effect (whether location or contamination) the variation within groups must be smaller than that between treatment groups. For example, Calvo-Bado *et al*. (2003) showed that the variation between treatment groups in their study of slow sand filters was much higher than the variation within a treatment group. As an evaluation of the DGGE method, it is reassuring that the largest degree of difference observed in this current experiment was between the pre-deployment substrate, originally from O'Brien Bay, and the hydrocarbon treatment deployed into Sparkes and Brown Bays. DGGE was thus able to distinguish between the treatments which would be expected to be most different: those subject to a different location and contamination.

Effect of hydrocarbon treatment

O'Brien Bay was the only location for which there was a significant difference between the control and hydrocarbon treatments (Tables II & III). This may be due in part to the wide variation in the control samples within each location. Another consideration is that DGGE only detects differences in composition and not absolute abundance. We did not measure bacterial abundance, but studies by Griffith *et al.* (1981, 1982) in the Arctic and studies in Antarctic soils have found that hydrocarbon contamination can stimulate the numbers of both hydrocarbon degrading and total heterotrophic bacteria (Delille 2000, Aislabie *et al.* 2001) or just the numbers of hydrocarbon degrading bacteria. Chemical analysis of the hydrocarbon treatments showed that biodegradation had occurred at all three locations, but significantly more so in Brown Bay (unpublished data). This would indicate that the microbial community in Brown Bay was more efficient at hydrocarbon biodegradation. It is also possible that the biodegradation occurred early in the incubation period, and by eleven weeks the degradable hydrocarbons were depleted. If hydrocarbon biodegradation were no longer a major process at Brown Bay the microbial community in the hydrocarbon treatment may have returned to a state that resembled the control treatment. As biodegradation was still occurring in O'Brien Bay, differences between the control and hydrocarbon treatments were still detectable. Although the control and hydrocarbon treatments in Sparkes Bay were not significantly different, there was some indication of a difference between them. In the MDS ordination there was some separation between them (Fig. 1f) and ANOSIM indicated a small difference (Table IId), suggesting that the microbial assemblage were in some way intermediate between the other two locations.

Effect of location

The results of the nested survey, involving replicated sampling at a series of nested spatial scales (Powell *et al.* 2003), indicated that pollution is one of a number of factors influencing sediment microbial populations in the Casey region. The results of this study provide more evidence that hydrocarbons have an effect on the sediment microbial communities, although the exact changes and the mechanisms by which they occur are yet to be elucidated.

The effect of the sediment deployment location on the microbial communities was only apparent in the presence of hydrocarbons. The largest effect of location was observed between the pre-deployment samples and the hydrocarbon treatments from Brown and Sparkes Bay. This may have been a result of the combination of the effects of location and hydrocarbon contamination. Pre-deployment communities were only marginally different from the Brown Bay control treatment and not significantly different from the O'Brien Bay treatments or the Sparkes Bay control (Tables II & III). As the sediment was originally collected from O'Brien Bay the similarity of the pre-deployment samples to the O'Brien Bay treatments is not surprising. The fact that the communities that developed in Sparkes and Brown Bay hydrocarbon treatments are different from the pre-deployment samples suggests that the location of deployment had an effect but only in the presence of hydrocarbon contamination. It is not possible to determine whether this effect was on the existing pre-deployment communities or on microbes recruited during the experiment.

The microbial populations in the hydrocarbon treatments from Sparkes Bay were significantly different from those in O'Brien and Brown Bay (Table II). It is likely that the hydrocarbons placed a selective pressure on the microbes, favouring those that are able to utilise hydrocarbons or their degradation products. As the natural microbial population in

Sparkes Bay is different from Brown and O'Brien bays (see Powell *et al.* 2003), the component capable of hydrocarbon degradation is probably also different. Similarly, as the natural microbial communities in Brown and O'Brien bays are similar (see Powell *et al.* 2003), it is not surprising that similar populations have developed in the hydrocarbon treatments in those two bays.

An effect of location in the comparison of the control treatments might have been expected if there was no influence from remnant populations and recruitment was the dominant source of microbes. Perhaps no differences were observed in the control samples because all the samples started with the same microbial population and diverse communities developed. This diversity resulted in large within-location variation that made differences between locations impossible to distinguish.

Although there have been several previous studies which demonstrated differences in the microbial populations of contaminated and pristine sites (Atlas 1981, Margesin *et al.* 2003), there are only a few studies which compare the effect of hydrocarbon pollution on pristine locations with the effects on previously contaminated sites, including one in Antarctic soil (Aislabie *et al.* 1998), one in Antarctic sea water (Yakimov *et al.* 2004) and two from temperate regions (Miethe *et al.* 1994, Olivera *et al.* 1997). However the focus of all these was on the potential for biodegradation rather than the effects on overall community structure. Our results suggest that previous contamination history is one of the factors that can effect the development of microbial communities in recently contaminated sediments.

Comparison with effects on benthic infauna and diatom communities

The hydrocarbon treatment had a different effect on the infaunal (Stark *et al.* 2003) and diatom (Cunningham *et al.* 2003) communities compared to the microbial communities. For the microbial communities an effect of the hydrocarbon treatment was only observed at O'Brien Bay. For the diatom communities an effect was observed at all three sites and for the infauna an effect of hydrocarbons was observed at Brown and Sparkes Bay. The lack of difference between control and hydrocarbon treatments in the recruitment of infauna to O'Brien Bay was attributed to the fact that the O'Brien Bay infauna is less stressed by existing pollutants. The diatom communities were most similar in O'Brien and Sparkes Bay whereas the microbial and infaunal communities were most similar in O'Brien and Brown Bay (see Powell *et al.* 2003, Stark *et al.* 2003).

The basis of the response of microbial communities to hydrocarbon contamination is fundamentally different from that of the diatoms and infauna. Only the microbes are capable of utilising hydrocarbons as a carbon source. Changes in community structure of the diatoms and infauna are most likely to be due either to species avoiding the contaminated sediment or to toxic effects. Changes in the microbial community are more likely to be due to the response of those species capable of utilising hydrocarbons. Regardless of the type of response, it is significant that all three trophic levels (bacteria, diatoms, and infauna) were affected by the hydrocarbon treatment.

Conclusions

The nature of our findings is similar to those reported for the few other comparable experiments that use DGGE to determine the effects of hydrocarbon contamination on microbial population structure (Macnaughton *et al*. 1999, Swannell *et al.* 1999, Rolings *et al.* 2004). That is, some treatment effects appear to be very obvious while other, more subtle, effects are more complex and less easily explained. Our results suggest that subtle differences between treatment groups may be obscured by the variability introduced by the PCR and gel stages in DGGE leading to a type II error (the null hypothesis of no significant difference is falsely retained). To avoid this it is important that care is taken to design experiments with enough samples and replicates of the DGGE analysis. To reduce variability introduced by the DGGE method when seeking subtle effects, comparisons between treatments are best done using samples that have been processed in the same round of PCR and the same gel run. To assist interpretation of future experiments of this type that use the DGGE procedure it is recommended that information be provided in the methods section on whether comparisons are among or within different PCR rounds and gel runs.

The location of deployment of the sediment can also have an effect on the microbial communities; although in our study this was only in the presence of hydrocarbon contamination. The design of future experiments of this type will need to take this into consideration if it is intended the results be used to make generalisations that can be applied to other locations rather than being site specific. The experiment must include replication at several locations if the results are to be extrapolated from one location to another. The practical implication of this to oil spill management is that hydrocarbon contamination may have a different effect on the microbial community depending on where it occurs and the contamination history of the site.

The variation in the DGGE banding patterns from multiple analyses of the same sample provided an indication of the diversity of the microbial community. The fact that different bands appear in each DGGE run implies that the species distribution is even and that the communities are not dominated by a few species. That this diverse microbial community is maintained even in the samples exposed to hydrocarbons suggests that hydrocarbon contamination at the concentrations used in this study is not highly toxic to the microbial community.

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