

PCR Analysis of Microbial Diversity in Brackish Water Microcosms

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Abstract

Microbial communities differing in biodiversity were established by inoculating sterile brackish water with serially diluted estuarine samples grown on media. Systems were perturbed by being driven anaerobic by the addition of glucose. Sulfate, nitrate, ammonia, dissolved oxygen and dissolved organic carbon of both microcosms were measured to monitor the microcosms' biogeochemical processes. PCR analysis was done on DNA samples that were preserved from the serial dilution and throughout the incubation to determine the effects of perturbation, and the effectiveness of serial dilution in decreasing the biodiversity of functional groups capable of nitrifying, denitrifying, reducing sulfate, and archeal and cyanobacteria. Serial dilution was determined to be a viable technique for reducing microbial diversity and changing community structure. Overall ecosystem function was not effected by changing levels of biodiversity, though differences were seen in the response to perturbation. The general mechanisms governing the differences in response appear to be the complementarity and selection effects.

Key Words: Serial Dilution, PCR, Biodiversity, Microbial Diversity, Functional Groups.

Introduction

Rising global temperatures, changing atmosphere, and reduced habitats have coincided with an increase of observed species extinction with more predicted in the near future (Loreau, 2000). However, beyond economic and

ethical reasons for the conservation of biodiversity and the maintenance of known species, it is not yet clear how biodiversity affects ecosystem function. The past decade has seen many experiments that attempt to address the issue, and while insights have been gained, there are no clear answers.

It has been shown that increased diversity yields greater primary production as well as greater resilience in recovery from perturbations like severe drought (Tilman et al, 1996, Loreau et al, 2001). The basic mechanisms that are theorized to lie behind these observed trends are the complementarity effect and the selection effect. The complementarity effect states that with additional species more functional niches are filled, yielding greater production and resilience. The selection effect states that environments with more species will select for those with extreme traits that can achieve dominance in the system (Loreau, 2000, Loreau et al, 2001). The vast majority of the work that has led to the development of these theories has focused on plant communities. Relatively few experiments have focused on the effects of biodiversity upon other trophic levels, identifying the exact functional niches of various species, or how changes in the diversity of one trophic level might affect other levels. Clarification of these issues, further development of theory, and greater understanding of the topic and its governing mechanisms are likely to arise from the results of experiments upon different systems.

Manipulations of bacterial biodiversity were only recently been undertaken in earnest. Most have focused on soil communities and have come to different conclusions through a variety of methods and experimental designs. Some found that the metabolic activity of bacteria is reduced with decreased diversity (Salonius, 1981) while others found increased metabolic diversity with lowered diversity (Ross et al, 2001), and some were not able find any consistent trend between a reduction in biodiversity and ecosystem function (Griffiths et al., 2000, 2001). The differences in the findings are likely due to unknown levels of microbial biodiversity, and the difficulty in eliminating all bacteria capable of performing a certain function. The varying results may also be a consequence of non-uniform measurements of diversity, a focus on the isolation of novel species

rather than the effects of diversity on ecosystem function, and the various paths taken to reduce microbial diversity.

The general methods used to test the diversity of bacterial communities consist of a combination of approaches. One is usually a molecular procedure like polymerase chain reaction (PCR), terminal restriction length polymorphism (T-RFLP), or denaturing gradient gel electrophoresis (DGGE) to ensure inclusion of the great number of species not seen in cultures. Community level physiological profiles (CLPP), and other culture-based measurements like colony morphology are also usually included in the experimental design. Interestingly, only Griffiths et al (2000,2001) and Saloniis (1981) have attempted to measure biogeochemical functions like respiration, nitrification and denitrification resulting from the different levels of biodiversity. With the importance of determining the functional characteristics of individual species becoming apparent in the study of biodiversity (Loreau et al, 2001, Griffiths et al, 2001), those measurements would seem to be a key to understanding the link between microbial biodiversity and ecosystem function.

Ways to experimentally alter biodiversity consists of destructive and constructive methods. Destructive methods like fumigation are effective, but tend to select for the survival of certain organisms rather than a random selection (Griffiths et al, 2001). A constructive method that has been utilized that has random selection of the species in the low diversity samples is serial dilution. Serially diluting a sample has proven to be a viable technique to reducing microbial diversity (Saloniis, 1981, Griffiths et al, 2000), changing community structure (Garland and Lehman, 1999, Franklin et al, 2000), and a method for isolating smaller cells that are numerically abundant in natural samples, but are out competed by other organisms in high nutrient cultures and as a result are often undetected (Morales et al, 1996, Jackson et al, 1998, De Fede et al, 2000). For these reasons, and the ease of applying the serial dilution technique to general biodiversity theories like the selection effect, we chose to use serial dilution as our technique to decrease microbial biodiversity.

Expanding from the scope of previous studies, we focused on the metabolic functions of the bacteria, and how they varied in high and low diversity brackish water microcosms. Another report details the findings of the biogeochemical processes that were measured in the microcosms (Charkoudian and Levine, 2001). For the testing of the presence of bacterial functional groups, PCR was chosen as the sole method. Due to the relatively low cost, ability to apply it to uncultivated natural samples, already developed primers that target specific functional groups, and relative ease of interpreting results it was chosen over the other possible molecular methods. CLPP and culture based measurements of biodiversity were not performed because only 2% of all microbial cells are expressed in culture (Griffiths et al, 2000), while PCR can amplify the presence of organisms that cannot be cultured (Jackson and Young, 2001).

Usually PCR results can only be used to tell the presence or absence of the targeted species or functional group, and it is generally accepted that an exact quantitative population composition from an environmental sample can never be confidently reported (Head et al., 1998, Muyzer and Ramsing, 1995). However, PCR on a time series with the same samples has rarely been performed, but previous studies were able to make conclusions about relative bacterial abundance based on the strength of the band by keeping the amount of template and primer DNA identical in all PCR reactions and assuming constant preferential amplification by the primers throughout the sample series (Teske et al., 1998).

An additional aspect of our experiment was monitoring the response of diversity and biogeochemical cycling to perturbation. Previous findings have indicated that, like plant communities, reduced microbial diversity recovered from stress slower than fully diverse communities (Griffiths, 2000). We hypothesized that serial dilution would eliminate functional groups of bacteria, and as a result the bacterial response to our stress, driving the system anaerobic, would vary between the low and high diversity microcosms. Insights would also be gained into the stability of a system, and how brittle the system would become when,

say, the functional group responsible for nitrification but not denitrification may be lost. Will and how might the system degrade or will function somehow be conserved? To answer these questions and test our hypothesis, brackish water from Child's River in Falmouth, MA was used to inoculate twenty liters of sterile water in a closed and dark system. The same sample serially diluted to 10^{-6} was used as the inoculum for the low diversity microcosm. Functional groups and bacteria tested for by PCR were sulfate reducing bacteria, denitrifiers, nitrifiers (ammonia oxidizers), archeals, and cyanobacteria. Sulfate, nitrate, ammonia, dissolved organic carbon, and dissolved oxygen was measured to monitor the biogeochemical processes of the systems and the functional groups for whose molecular presence were being tested.

Methods:

Serial Dilution.

Brackish water (11 ppt) was acquired from Child's River in Falmouth, MA, and 10 mL were serially diluted to 10^{-6} of the original sample. The fully diverse sample, 10^{-4} , 10^{-5} and 10^{-6} dilutions were grown on Reasnor-Geldrich (RG) media (Charkoudian and Levine, 2001). Samples were preserved for DNA extraction and PCR analysis before they were used as inocula for the microcosms and once their bacterial abundance, as measured by DAPI staining, had reached more than 7×10^7 .

Incubation Experiment.

The fully diverse sample and the 10^{-6} dilution grown on media were used as inocula, respectively, for the fully diverse and low diversity microcosms. The microcosms were 20L carboys filled with autoclaved Child's River water, augmented with nutrients, and kept closed and in the dark (Charkoudian and Levine, 2001). Sampling of the fully diverse microcosm (F1) and the low diversity microcosm (L1) took place shortly before the addition of glucose, 12-24 hours after becoming anaerobic, 6 days after becoming anaerobic, and at the conclusion of the experiment. The low diversity microcosm was also sampled

two days after the addition of glucose, but two days before it became anaerobic. Sampling points are visually represented in Figures 1 and 2. Replicate low and full diversity microcosms were also established, but were not sampled for PCR analysis due to the time constraints of this study.

DNA Sample Preservation.

The first samples that were collected for DNA extraction was from fully diverse water from Child's River in Falmouth, MA. The water was put into a 1 L bottle and a handful of sediment was thrown into the bottle. Immediately upon

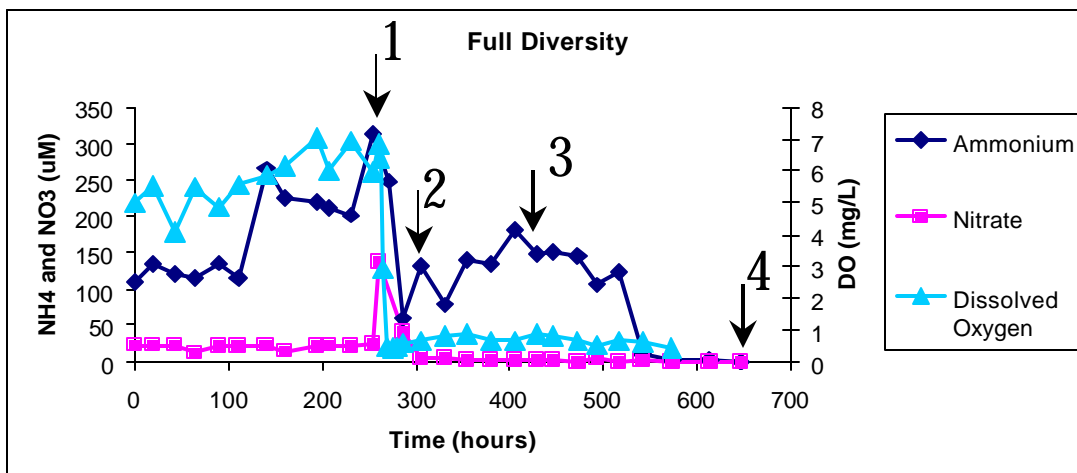


Figure 1. The concentration (uM) of ammonium and nitrate and the amount of dissolved oxygen (mg/L) in the fully diverse microcosm over time (hours). The arrows represent when samples from the microcosms were concentrated onto filters to facilitate DNA extraction and PCR analysis. Numbers correspond to the lanes which the samples' PCR products were run on a 1% agarose gel. (See Charkoudian and Levine, 2001)

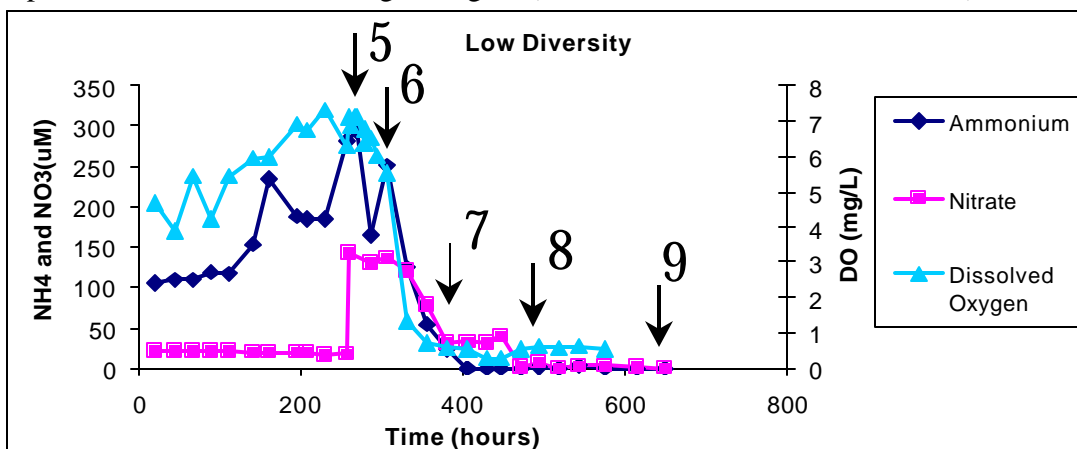


Figure 2. The concentration (uM) of ammonium and nitrate and the amount of dissolved oxygen (mg/L) in the low diversity microcosm over time (hours). The arrows represent when samples from the microcosms were concentrated onto filters to facilitate DNA extraction and PCR analysis. Numbers correspond to the lanes which the samples' PCR products were run on a 1% agarose gel. (See Charkoudian and Levine, 2001)

returning to the lab, approximately 400 mL was concentrated onto a 0.2µm Sterivex filters (Millipore). All water was forced out the filter with air and the filter was then capped and immediately flooded with ~2 mL of DNA extraction buffer (0.1 M Tris-HCL (pH 8.0), 0.1 M NaEDTA (pH 8.0), 1.0 M Na₂H₂PO₄ (pH 8.0), 1.5 M NaCl, 0.5% Hexadecylmethylammonium bromide (CTAB) and 10% sterile H₂O), and frozen for further processing at -80°C. Between 120 and 500 mL of all samples were concentrated in the same manner.

DNA Extraction.

The DNA was extracted from the cells concentrated on the Sterivex filter following protocol adapted from Zhou et al. (1996). The filters were thawed and 20µL of Proteinase-K (10% solution in water) was added. The filters were then thawed while slowly rotating at 37°C and frozen at -80°C three times. They were then incubated at 37°C for a half hour at which point 100µL of sodium dodecyl sulfate (SDS, 20% solution in water) was added, and the filters were incubated for 2 hours while slowly rotating at 65°C. The buffer was then withdrawn from filter and it was flooded again with ~2ml of DNA extraction buffer (DEB), and incubated while rotating at 65°C for 30 minutes. The DEB wash was withdrawn and combined with the previously withdrawn buffer. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added, and the sample was vortexed for 10 seconds. After centrifuging for 5 minutes at 2000 RPM in a clinical centrifuge, the entire top layer was removed and the centrifugation was repeated with the subsequent removal of the top layer. Isopropanol was added at 60% of the total volume and incubated at room temperature overnight. The samples were then centrifuged at 22°C at 15,800xG for a half hour. The buffer was then poured off from the pellet, and washed with 70% Ethanol and centrifuged 15,800xG for five minutes. The pellet was then resuspended with 500µL of Elution buffer (EB, 10mM Tris-Cl, pH 8.5) from Qiagen. Half of the sample (250µL) was stored at -80°C, while the other half was stored at -20°C for immediate use.

Primer Selection.

All of the primer pairs used in this study have been shown to have relatively good specificity for their targeted functional groups, and have been used successfully with environmental samples (Crump et al, 1999, Kowalchuk et al., 1997, Amann et al., 1992, West and Scanlan, 1999, Braker et al., 1998, Amann et al., 1995). All the primer pairs target 16S genes except for the one targeting denitrifiers. Due to the phylogenetically diverse nature of denitrifiers, it is not feasible to use primers that target 16S DNA to detect them (Ward, 1995). Instead, primer pairs were used that target nitrite reductase genes (specifically *nirS*), and which were shown to successfully detect denitrifying bacteria in environmental samples (Braker et al., 1998). Sequences and identity of the primer pairs used in this study are given in Table 1.

The primer pairs used in this study were based on the different species that are important in the biogeochemical processes that we monitored and selected for in the addition of certain nutrients, and in driving the system anaerobic. We hypothesized that any difference between the levels of nitrate,

Table 1. PCR primers used in this study, their sequences, targeted group, amplification conditions and number of cycles used, and the reference from which sequence and temperature conditions were taken from.

Primer	Sequence (5'-3') of Primer	Target Group	PCR Conditions	Reference
8F 1492T	ATRGTTTGATCCTGGCTCA CGCCAACTTCTTAACACT	Universal Bacteria	25-30 cycles, 58.5 C	Crump et al. (1999)
CTO 189C CTO 265T	GGAGGAAAAGTAGGGGATCG CTACCGTGTGTCACAT	Ammonia - Oxidizers	Nested PCR with Gen. Bact PCR: 20 cycles, 56.5 C	Kowalchuk et al. (1997)
385 F 907R	CGGCGTGCCTTCAGG GCGCAATGCTTTCATG	Sulfate Reducers	20 cycles, 63-53 C Touchdown 10 cycles, 53 C	Amann et al. (1992)
CYA359F 1492T	GGGGAATYTTCCGCAATGGG CGCCAACTTCTTAACACT	Cyanobacteria	30 cycles, 50.7 C	West and Scanlan (1999)
4f 1492T	TCCGGCRGGATCAACCGGAA CGCCAACTTCTTAACACT	Universal Archaeal	35 cycles, 53.6 C	Amann et al. (1995)
nirS1f (763-780) nirS6r (1638-1653)	OCTAYTGCGCGCCRCART CGTTGAACTTRCCGGT	Denitrifiers	20 cycles, 53-43 C Touchdown 10 cycles, 43 C	Braker et al. (1998)

PCR Amplification and Visualization.

PCR reactions were performed using an Eppendorf Mastercycler Gradient. All reactions were done in a total volume of 25^oL, with 10x PCR buffer (100 mM Tris, 15 mM MgCl₂, 500 mM KCl, 1% Triton), 10x (20mM) deoxynucleotides, and 1 U Taq polymerase. In each reaction 25ng of each primer and between 10-20 ng of template DNA was used. To facilitate comparisons of the differences between the samples, the concentration of DNA template in every time series and dilution comparison was identical. All PCR reactions began with an initial denaturation at 94^oC for five minutes, followed by cycles of 30 second denaturing at 94^oC, annealing at temperatures specific for each primer pair (Table 1), extension at 72^oC for 3 minutes, and a final extension at 72^oC for 5 minutes. All reactions were run with a negative control consisting of sterile water instead of template DNA.

PCR products were visualized using gel electrophoresis. Bromophenol blue loading dye added to 10 μ L of each sample, and 4 μ L of a 1 kB ladder was loaded into a 1% agarose gel stained with ethidium bromide. All gels were run at 93 volts for 25-30 minutes. Gels were immediately photographed using an Ultralum digital camera.

Results

The ammonia oxidizers (nitrifiers) were diluted out as no band of the expected size was present in any of the dilutions or the low diversity microcosm's inoculum, the 10⁻⁶ dilution (Figure 3). Anaerobic conditions did not effect the the presence of ammonia oxidizers in the fully diverse sample, though the addition of glucose appears to have resulted in their brief presence in the low diversity sample, despite an absence in the inoculum (Figure 4). Prolonged anaerobic conditions once again caused their absence.

All of the archeals were diluted out, and it appears that they did not grow on the RG media as the band was much lighter (Figure 5). The results of the time series also indicates that the archeals could not compete in the microcosms'

environments as they are not present in the fully diverse microcosm despite being in the inoculum, and their presence does not return even under prolonged anaerobic conditions (Figure 6).

Sulfate reducing bacteria were not diluted out as they are present in both the fully diverse inoculum and the low diversity inoculum at the expected size of approximately 500 base pairs (Figure 7). The sulfate reducing bacteria appear to be unaffected by the changing conditions as they are present throughout the course of the experiment. The unexpected band of approximately 1000 base pairs is likely to be non-specific binding despite stringent PCR conditions. Of note, this was the only primer pair that showed a positive result in the negative control (results not shown), but the band, while not very strong, was still darker than some of the results seen from the natural samples so it seems that despite contaminated primers the results are viable.

The denitrifiers were also not diluted out (Figure 8), but its species composition changed throughout the course of the experiment and time series (Figure 9). Though only sequencing will confirm the presence of different species, the novel bands that are consistently seen above the expected product size of 890 base pairs do not appear to be the result of non-specific binding. They were reproduced several times (results not shown) under increasingly stringent PCR conditions. As the system became more anaerobic and nitrate was consumed the novel species' presence disappeared.

Cyanobacteria were present in both inocula (Figure 10), and throughout

Table 2. Key to figures of PCR products.

Lane	Sample
A	Fully diverse Child's River sample not grown on media
B	Fully diverse sample grown on RG media and what was used as the inoculum for the fully diverse microcosm.
C	10 ⁻⁴ dilution grown on RG media
D	10 ⁻⁵ dilution grown on RG media
E	10 ⁻⁶ dilution grown on RG media, and what was used as the low diversity microcosm inoculum.
1	Fully diverse microcosm before the addition of glucose.
2	Fully diverse microcosm 12 hours after becoming anaerobic.
3	Fully diverse microcosm 6 days after becoming anaerobic.
4	Fully diverse microcosm at the conclusion of the experiment.
5	Low diversity microcosm before the addition of glucose.
6	Low diversity microcosm 2 days after the addition of glucose.
7	Low diversity microcosm 24 hours after becoming anaerobic.
8	Low diversity microcosm 6 days after becoming anaerobic.

the time series (Figure 9) indicating remarkable resiliency in unfavorable conditions.

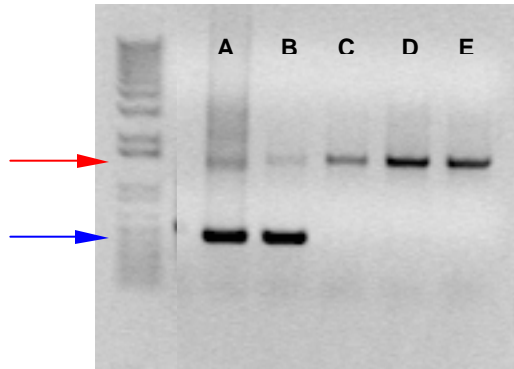


Figure 3. Nested PCR results of the serial dilution using the primers for ammonia oxidizers, CTO 189fC and CTO 654 r. The unexpected higher bands indicated by the red arrow are a result of amplification from the 8f and 1492 universal bacteria primers that were still in the PCR product used as the template in the nested reaction. For key to lane assignments see Table 2.

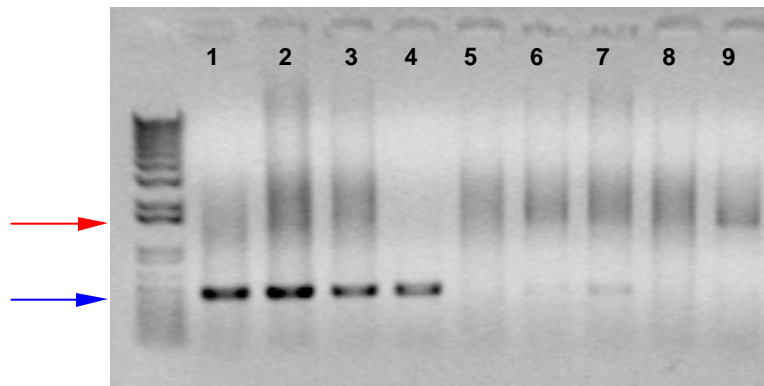


Figure 4. Nested PCR results of the time series using the primers for ammonia oxidizers, CTO 189fC and CTO 654 r. The unexpected higher bands indicated by the red arrow are a result of amplification from the 8f and 1492 universal bacteria primers that were still in the PCR product used as the template in the nested reaction. For key to lane assignments see Table 2.

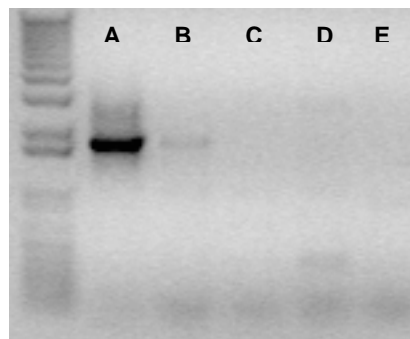


Figure 5. PCR results of the serial dilution using the Universal Archeal primers, 4f and 1492r. For key to lane assignments see Table 2.

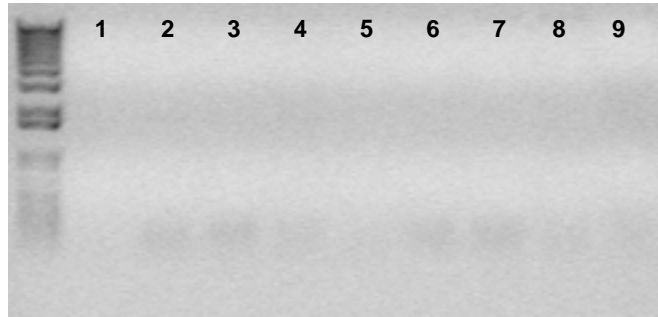


Figure 6. PCR results of the time series using the primers for Universal Archeal primers, 4f and 1492r. For key to lane assignments see Table 2.

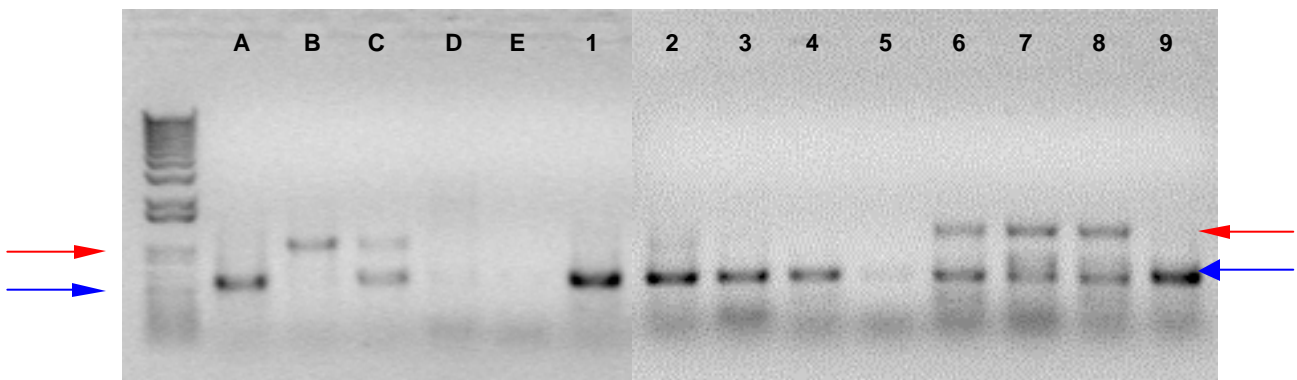


Figure 7. PCR results of the serial dilution and time series using primers 385f and 907r to detect the presence of sulfate reducing bacteria. For key to lane assignments see Table 2. The expected band size is indicated by the blue arrow, while the red arrow indicates unexpected bands.

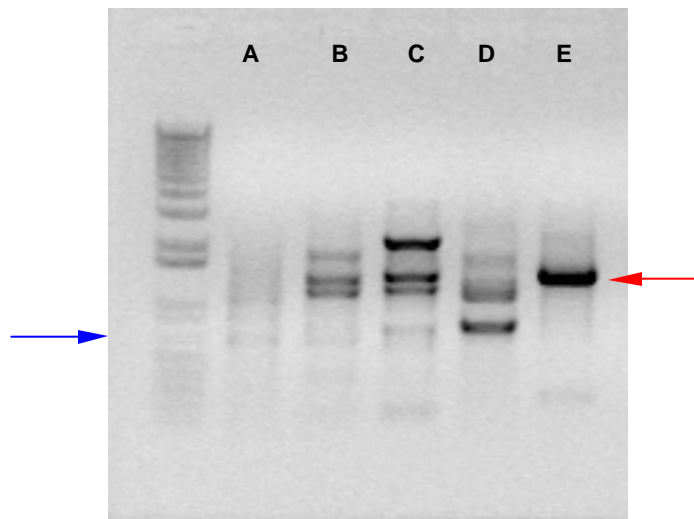


Figure 8. PCR results of the serial dilution using the primers nirS1f and nirS6r to test for the presence of denitrifiers. For key to lane assignments see Table 2. The expected band size is indicated by the blue arrow, while the red arrow indicates unexpected bands of particular note.

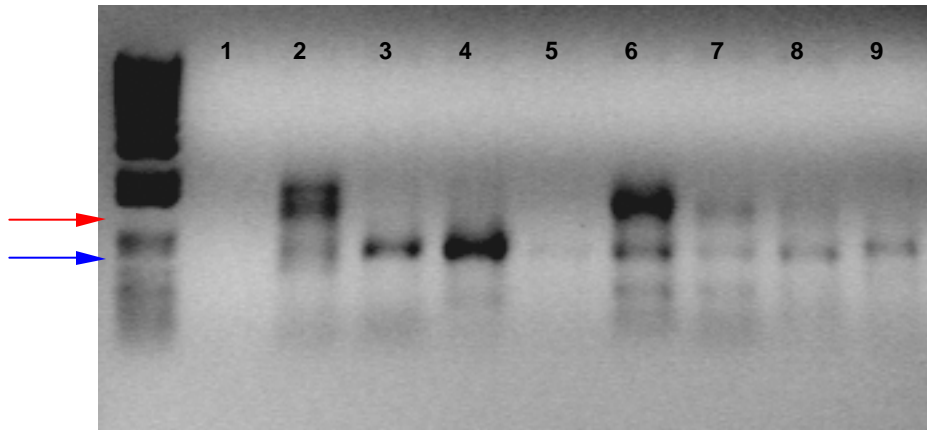


Figure 9. PCR results of the time series using the primers nirS1f and nirS6r to test for the presence of denitrifiers. For key to lane assignments see Table 2. The expected band size is indicated by the blue arrow, while the red arrow indicates unexpected bands of particular note.

Discussion

Serial dilution was determined to be a viable technique for reducing microbial diversity and changing community structure, confirming earlier findings (Salonius, 1981, Garland and Lehman, 1999, Franklin et al, 2000, Griffiths et al, 2000). The diluting out of ammonia oxidizers (Figure 3) as well as all archaeals (Figure 5) was a clear indication of the decreased diversity of the low diversity microcosm. The clear presence of ammonia oxidizers in every fully diverse sample throughout the time series (Figure 4) and in the inoculum, as compared with the general absence of the ammonia oxidizers in the low diversity microcosm was the result of the serial dilution. The reappearance of very faint bands of ammonia oxidizers in the low diversity microcosm after the addition of glucose is difficult to explain. It is not expected nor logical that the onset of anaerobic conditions or the addition of an organic carbon source would cause a boom in the remaining population of these chemolithoautotrophs. Their absence after prolonged anaerobic conditions was expected, though with their presence maintained in the fully diverse sample throughout the time series it is curious that the population of the ammonia oxidizers in the low diversity microcosm would appear and then subsequently disappear. Nevertheless, the differences between the microcosms are apparent.

While it is not clear whether the elimination of the archeals was a result of serial dilution, its decrease in intensity in the fully diverse inoculum compared with the natural sample, and its absence in the fully diverse microcosm throughout the course of the experiment indicate that they were selected against by the conditions and environments established in the experiment. The experimental conditions seem to have played a greater role in affecting archeal populations as compared to the serial dilution as even after prolonged anaerobic conditions in the fully diverse microcosm, which was expected to have selected for archeal bacteria like methanogens, the population remained absent.

Unfortunately, both of these results which could have confirmed our initial hypothesis that the absence of functional groups would have changed biogeochemical processes was not observed (Figures 1 and 2, Charkoudian and Levine, 2001). In the case of the archeals, measurement of the biogeochemical processes that they perform, such as methanogenesis, were not included in the experimental design. Similarly, there was a lack of ammonia oxidization in the fully diverse microcosm despite their clear genetic presence.

It is possible that both groups were not given enough time to grow in the environment, and in future experiments more time should be allowed for the communities to change, grow, or become active so that difference between treatments can definitely be seen.

The continual presence of cyanobacteria in both the full and low diversity microcosms (Figures 10 and 11) is an indication that they are remarkably resilient. While the experimental conditions were designed to select against cyanobacteria, they were able to maintain their presence throughout the experiment despite being in the dark and in anaerobic conditions. It is noteworthy that the PCR technique is unable to whether cells are in an active or inactive state, and it is likely that the cyanobacteria were dormant.

Unfortunately, the 385f primer used to test for the presence for sulfate reducing bacteria is selective but not specific, and as such can only be appropriately used qualitatively (Amann et al., 1992). Thus, even though PCR conditions were optimized to allow for a quantitative estimate of the time series

and serial dilution, such conclusions are inappropriate for this primer pair. The novel band at approximately 1000 base pairs that is common in many of the samples is likely to be the result of non-specific binding despite the stringent PCR conditions because of the inaccuracies of the primer pair. Sequencing of the band would still be justified, as it is conceivable that it is amplifying a novel sulfate reducing bacteria with multiple copies of the 16S gene. Interestingly, much like the ammonia oxidizers, there was no detectable change in sulfate reduction in either microcosm despite the genetic presence of the bacteria and the anaerobic conditions and nutrient additions designed to accelerate their growth (Charkoudian and Levine, 2001).

Clear changes in community structure and function resulting from the dilution and perturbation is seen in the PCR results from the denitrifiers (Figures 8 and 9), and the biogeochemical data (Figures 1 and 2). Unlike the primers for sulfate reducers, the primers for denitrifiers are highly specific and target the functional gene *nirS* rather than a 16S rDNA gene (Braker, et al. 1998, 2001). *NirS* has been found to have high levels of diversity in natural samples, and novel marine *nirS* genes were recently found in Puget Sound (Braker, et al. 2001). Unlike with the primer pair for sulfate reducing bacteria, the characteristics of the *nirS1F-nirS6R* primers leads to the ability to make rough conclusions about the relative bacterial abundance in the sample, as well as the conclusion that the novel band consistently seen at approximately 1500 base pairs is one of a novel species. It is likely, based on our data and previous findings about the effect of serial dilutions (Morales et al, 1996, Jackson et al, 1998, De Fede et al, 2000), that this band is a more generalist bacteria that is capable of denitrifying. Usually it would be out competed in a natural environment by specialists (the expected band size, 890 base pairs is based on Braker, et al.'s (1998) work testing the primer pair on cultures of known denitrifiers and testing of its effectiveness on natural samples), but growth on RG media and dilution selected for its expression. This is displayed in Figure 8 as the novel band appears with growth on media in lane 1, and its presence is strongest with the greatest dilution (lane 5).

The conclusion that the novel band is a generalist is supported by the data from the denitrifiers' time series (Figure 9). Before the addition of glucose in both samples (lanes 1 and 5), both bands are either very faint or absent. Once glucose and nitrate were added the conditions for denitrifying bacteria was ideal, and both bands are present with the novel species out competing the specialist (lanes 2 and 6). This is particularly true in the low diversity sample where it was originally present in greater abundance. As the microcosms become anaerobic and the levels of nitrate are diminished, the specialist is once again able to out compete the generalist. These conditions are likely to be more similar to those found naturally, and explains why the novel band was not seen in studies using the nirS1F-nirS6R primer pair on natural samples (Braker et al, 1998).

Further support for the increased presence and selection for the generalist is seen in the biogeochemical data. The delay in the low diversity microcosms becoming anaerobic and the subsequent delay in the decrease of nitrate levels after the addition of glucose as opposed to the almost immediate response of the fully diverse microcosm (Figures 1 and 2) can be explained by the inefficiency of this and other generalists to respond as quickly or as effectively as the specialists.

These findings indicate that the theoretical basic mechanisms behind the diversity responses of plant communities also hold true in the governing the response of low and full diversity bacterial communities. Complementarity and the selection effect are both seen operating behind the efficient response to the glucose addition. With greater numbers of species present, more functional niches were occupied and there was a faster response. Also, the more diverse system selected for species with extreme traits that were able to achieve dominance and respond expeditiously to the glucose addition. However, the overall functions of the system were unchanged. The nitrate and dissolved oxygen levels, though delayed, did eventually decrease to the same levels as was in the fully diverse microcosm. Thus, we reach the same conclusion as Griffiths et al. (2000, 2001) that there is no consistent trend between a reduction

in biodiversity and ecosystem function, though resilience to perturbation is reduced in low diversity samples.

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