The Effects of Microbial Diversity on Biogeochemical Processes in Brackish Water Microcosms

LOUISE K. CHARKOUDIAN[1]
URI LEVINE[2]

[1]*Haverford College, Department of Chemistry, Haverford, PA 19041*
[2]*Kenyon College, Department of Biology, Gambier, OH 43022*

Abstract

A microcosm experiment was performed to determine the effects of microbial biodiversity on biogeochemical processes in brackish water systems. Microbial communities of full and low diversity were established by inoculating heat-sterilized estuarine water with undiluted and serially diluted brackish microbial communities. The microcosms were acclimated, and then driven anaerobic upon the addition of high concentration carbon substrates. Dissolved oxygen, ammonium, nitrate, sulfate and dissolved organic carbon concentrations were monitored to determine the effect of biodiversity on the community biogeochemical processes. During certain periods, both full and low diversity microcosms did not exploit the environmental resources. The low diversity microcosm exhibited a delay in response to the addition of carbon sources and exhibited sub-optimal energetic metabolism.

Key words: microbial diversity, serial dilution, non-equilibrium thermodynamics

Introduction

Microbes have been evolving for nearly four billion years, are capable of exploiting a vast range of energy sources, and thrive in almost every habitat. For two billion years microbes were the only form of life on earth (Madigan et al. 2000). During this time, all basic biochemistries evolved and life forms developed from microbial

ancestors. Microorganisms represent the richest repertoire of molecular and chemical diversity in nature and they underlie basic ecosystem processes, such as biogeochemical cycles. Microbes participate in global cycles of elemental fixation and release, energy generation and consumption, and synthesis, degradation and recycling of complex chemicals and materials.

Several redox reactions of nitrogen are carried out in nature exclusively by microorganisms. High energy is required to break the triple bond of thermodynamically stable molecular nitrogen gas, which is the principal form of nitrogen on Earth. Nitrogen-fixing bacteria are the only organisms capable of converting this large reservoir of nitrogen to available forms, such as ammonia; recently, anthropogenic nitrogen fixation has surpassed natural nitrogen fixation. Nitrifying bacteria can oxidize ammonia to produce nitrite then nitrate, which can be used as an alternative electron acceptor and converted into gaseous nitrogen compounds by denitrifying bacteria.

Bacteria also play major roles in the chemical and biological redox reactions that make up the sulfur cycle. Sulfur and sulfide-oxidizing bacteria produce sulfate, which is used by sulfate reducing bacteria as an alternative electron acceptor in anaerobic respiration to produce hydrogen sulfide. Sulfate-reducing bacteria cycle hydrogen sulfide through the environment for use by anaerobic photosynthetic bacteria and sulfur-oxidizing bacteria, while returning carbon dioxide to the atmosphere.

The fusion of microbial ecology and industrial microbiology offers the possibility of degrading byproducts of increasing population growth and industrialization. Microbial metabolism may be applied to remove nitrogenous compounds from industrial and municipal wastewater through coupled nitrification-denitrification. Bioremediation uses microbial transformations to convert harmful substances to non-toxic compounds. Microbial communities treat widespread subsurface level contaminations by the oxidation, reduction or volatilization of metal and radionucleotide species such as chromium, mercury, arsenic, selenium, iron, manganese, technetium, uranium and plutonium (Jackson et al. 2001). Bioremediation exploits the diversity and genetic versatility of microorganisms for transformation of contaminants into less-harmful end-products, which are then integrated into natural biogeochemical cycles. Successful use of microbes for bioremediation depends on understanding the environmental factors, such

as biogeochemical cycles, responsible for the maintenance of this biodiversity and the ability to manipulate the diversity to improve and stimulate degradation rates and extents in the environment (Pritchard et al. 1995).

The conservation of biodiversity is of considerable importance because of the role community composition plays in maintaining ecosystems under changing conditions (Wardle et al. 1999). It has been concluded that the capacity of soil microbial systems to resist changing conditions is related positively to species numbers, and diversity provides insurance against large changes in ecosystem processes (Griffiths et al., 2000). Biodiversity experiments have been dominated by experiments concerned with above ground organisms (Giffith et al. 2000). The effects of biodiversity on microbial ecosystem processes may differ from those occurring aboveground, as it is generally thought that there may be considerable overlap in function at the level of microbial species (Chapin et al. 1997). It has also been speculated that high biodiversity may be vitally important in structurally diverse ecosystems (Grime 1997), such as estuarine sediments. The loss of species with similar functional roles, but differing in environmental response may reduce the resilience of ecosystems to stress (Chapin et al. 1997). It is therefore important to study biodiversity under both stable and non-stable conditions.

A key to establishing empirical relationships between biodiversity and ecosystem processes is the manipulation of species diversity in model systems. This has generally been achieved constructively through the addition of serially diluted communities (Gerland et al. 1999; Griffith et al. 2001). The premise behind this technique is that dilution of a diverse community will remove rare organisms, creating mixtures of cells differing in species richness (Franklin et al. 2000). Regrowth of the diluted mixtures should produce cultures of same biomass but different diversity. The serial dilution technique has been used to study relative structural diversity in mixed communities (Garland and Lehman 1999), the response of pasture soil communities to fumigation-induced microbial diversity reductions (Giffith et al. 2000), and the impact of dilution on structure and function in sewage microbial communities (Franklin et al. 2000).

These previous microbial biodiversity studies have focused on terrestrial communities and little attention has been given to aquatic environments. Moreover, few studies have examined impacts of microbial diversity on biogeochemical cycles.

Diverse microbial communities are capable of using the most energetic reactions in their environment. If all biogeochemical niches are satisfied, then non-equilibrium thermodynamics may be used to describe and predict biogeochemical processes of a community (Vallino et al. 1996). Low diversity communities, however, may not have the assortment of bacteria necessary to fill all niches and allocate their resources to optimize energetic metabolism. Thus, low diversity microcosms may exhibit behavior that is not well predicted by non-equilibrium thermodynamic models.

The present experiment uses the serial dilution technique to examine the relationship between microbial biodiversity, biogeochemical function and stability in brackish water microcosms. Microbial communities of full and low diversity were established by inoculating heat-steriled estuarine water with undiluted and serially diluted (10^{-6}) brackish microbial communities. The batch cultures were regrown to similar biomasses and acclimated to the heat-sterilized, nutrient supplemented estuarine water. The microcosms were driven anaerobic upon the addition of dextrose, acetate and lactate. Throughout the experiment, dissolved oxygen, ammonium, nitrate, sulfate and dissolved organic carbon concentrations were monitored. The results were analyzed to conclude the effects of biodiversity on the community biogeochemical functional processes and stability to perturbation.

Methods

Salinity was measured along Child's River (Falmouth, MA) using a refractometer. 100 Liters of 11ppt water was collected using five 20-liter Nalgene® High Density Polyethylene carboys. Two liters of 11ppt Child's River water and a handful of sediment were collected in two 1-liter Nalgene® bottles and refrigerated to be used for the inoculum in the microcosm experiment. The 100 liters of water were autoclaved in 2-liter Erlenmeyer flasks at 121°C for 35 minutes each. Four 20-liter Nalgene® High Density Polyethylene carboys were filled with the heat-sterilized water. Ammonium

sulfate (50µM) and 4µM potassium phosphate were added to each carboy as nutrient supplements.

The sample was serially diluted using 10mL of the Child's River inoculum sample (10^0) and diluting it ten-fold into 9mL of sterile Child's River. The dilution was carried out to the 10^{-6} dilution in a sterile laminat flow hood. The 10^0, 10^{-4}, 10^{-5} and 10^{-6} dilutions were added to separate 1-liter 0.22uM Millipore Express® sterile filter units, each containing 900mL of filtered heat-sterilized Child's River water and 100mL of Reasnor-Geldrich medium (0.5grams protease peptone, 0.5grams yeast extract, 0.5 grams casamino acids, 0.5 grams dextrose, 0.5 grams starch, 0.3 grams potassium phosphate, 0.05 grams magnesium sulfate and 0.3 grams sodium pyruvate). Bacterial abundance in each dilution was monitored throughout the incubation of the inoculum using DAPI counts (procedure modified from Feig and Porter 1980). Once the undiluted inoculum grew up to 10^7 bacteria per mL, 300mL of the inoculum was added to two of the carboys. The bacterial abundance of the diluted inoculum was monitored until the lowest dilution (10^{-6}) grew up to 10^7 bacteria per mL, at which point 300mL of the inoculum was added to the remaining two carboys. A control was run to guarantee sterile technique throughout the serial dilution and incubation of the inoculums.

Samples were taken from each carboy for gram stain tests, which differentiates bacteria into two categories based on cell wall structure (Protocol ™, Biochemical Science, Inc), and for PCR analysis. After 140 hours of acclimation, an additional 50µM ammonium sulfate was added to each carboy to stimulate nitrifying bacteria. The microcosms were driven anaerobic after 260 hours upon the addition of 1mM dextrose. At this time, the microcosms were supplemented with 100µM potassium nitrate. After 450 hours, 1mM acetate was added, then after 520 hours 3mM lactate and 3µM potassium phosphate were added to stimulate acetate oxidizing (group II) and non-acetate oxidizing (group I) sulfate-reducing bacteria. The microcosms were buffered with 5mM sodium bicarbonate after 300 hours to maintain the optimal pH for microbial growth of 5-9 (Madigan 2000) (Appendix A).

Dissolved oxygen, pH and bacterial abundance were monitored every 24 hours. Bacterial abundance was monitored using DAPI counts (Fieg 1980). DAPI count samples were preserved with 50% gluteraldehyde and analyzed using a Zeiss Universal®

microscope. Samples were taken from each carboy every 24 hours and filtered through ashed Whatman® GF/F filters for ammonium, nitrate, dissolved organic carbon (DOC) and sulfate analysis. Ammonium samples were preserved with 50µL 5N HCl, kept at 4°C, and then run on the Shimadzu® 1601 spectrophotometer (standard spectrophotometric method modified from Striekland, 1972). The samples were run at a 1:5 dilution using a standard curve acidified with 1µL 5N HCl/mL standard. Frozen nitrate samples were thawed and run on the Quikchem Lachat 8000® using the Automated Flow Injection Analyzer. DOC samples were acidified with 100 µL of 43% phosphoric acid, sparged with oxygen gas to remove carbon dioxide and other combustion gasses, and then kept at 4°C. DOC samples were injected into a high temperature catalytic oxidation (HTCO) machine (method adapted from Peltzer 1993), which was calibrated using glucose standards. Frozen sulfate samples were thawed and run on the Quikchem Lachat 8000® using ion chromatography. Hydrogen sulfide concentrations were measured at the end of the microcosm experiment using a spectrophotometric method adapted from Gilboa-Garber (1971).

Results:

The experiment was designed to give two replicates of the full diversity and low diversity microcosms. The trends in the carboys replicated well (Appendix B) and mean values will be referred to throughout the remainder of the text.

The full diversity microcosms contained both gram-positive and gram-negative bacteria, whereas the low diversity microcosms only contained gram-negative bacteria. PCR analysis revealed the presence of nitrifiers, universal Archeal, cyanobacteria, denitrifiers and sulfate reducers in the full diversity microcosm, and the presence of cyanobacteria, denitrifiers and sulfate reducers in the low diversity microcosm (Levine and Charkoudian, 2001).

Bacteria in the full diversity inoculum grew up to 10^7 bacteria per mL in the Reasnor Geldrich medium within 24 hours. Bacterial abundance in the 10^{-5} dilution reached 10^7 bacteria per mL after 35 hours. After 50 hours, the 10^{-6} dilution grew up to 10^7 bacteria per mL. Within 24 hours after inoculation of the 20-liter carboys, both the

full diversity and the low diversity microcosms had grown up to 10^6 bacteria per mL (Figure 1). Bacterial abundance increased to 10^7 bacteria per mL upon addition of dextrose. The full diversity microcosm went anaerobic eight times faster than the low diversity upon the addition of dextrose (Figure 2).

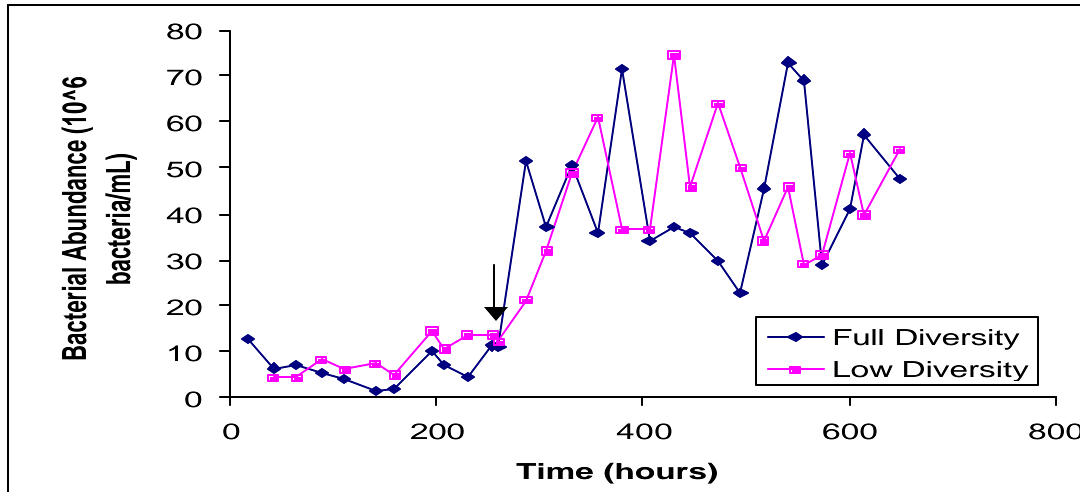

Figure 1 Bacterial abundance in full and low diversity microcosms. Arrow represents the addition of 1mM dextrose at 260 hours.

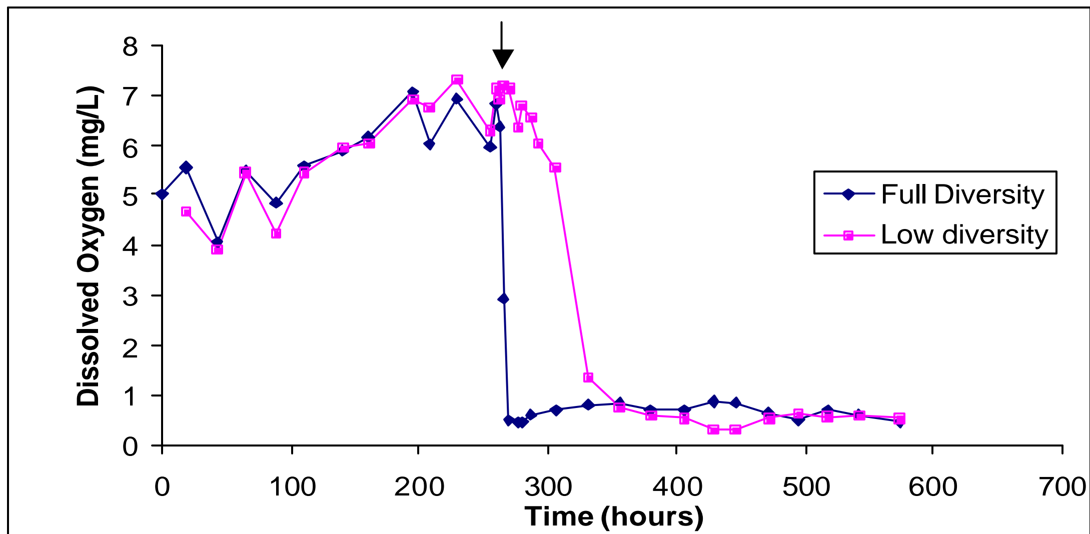

Figure 2 Concentrations of dissolved oxygen in full and low diversity microcosms. Arrow represents the addition of 1mM dextrose at 260 hours.

Neither the full diversity nor low diversity microcosms used up ammonium via nitrification during the first 150 hours of the experiment (Figure 3 and Figure 4) even though nitrifiers were present in the full diversity treatments (Levine and Charkoudian 2001). Within 30 hours after the addition of dextrose, the ammonium levels of the low

diversity microcosm decreased by 116µM. The decrease in ammonium level during this period in the full diversity microcosm was two times greater. The ammonium concentrations in both microcosms increased by about 90µM upon the addition of the potassium nitrate and by 75µM sodium bicarbonate buffer. The increase in ammonium concentrations due to the addition of potassium nitrate and sodium bicarbonate is most likely due to a matrix effect. The potassium nitrate and sodium bicarbonate are components of the sample other than the analyte, which interfere with the measure of ammonium quantity (IUPAC 1997). Thus, the peaks present in the ammonium data at 260 hours and 307 hours are most likely not due to an increase in ammonium, but rather the interference of the additions.

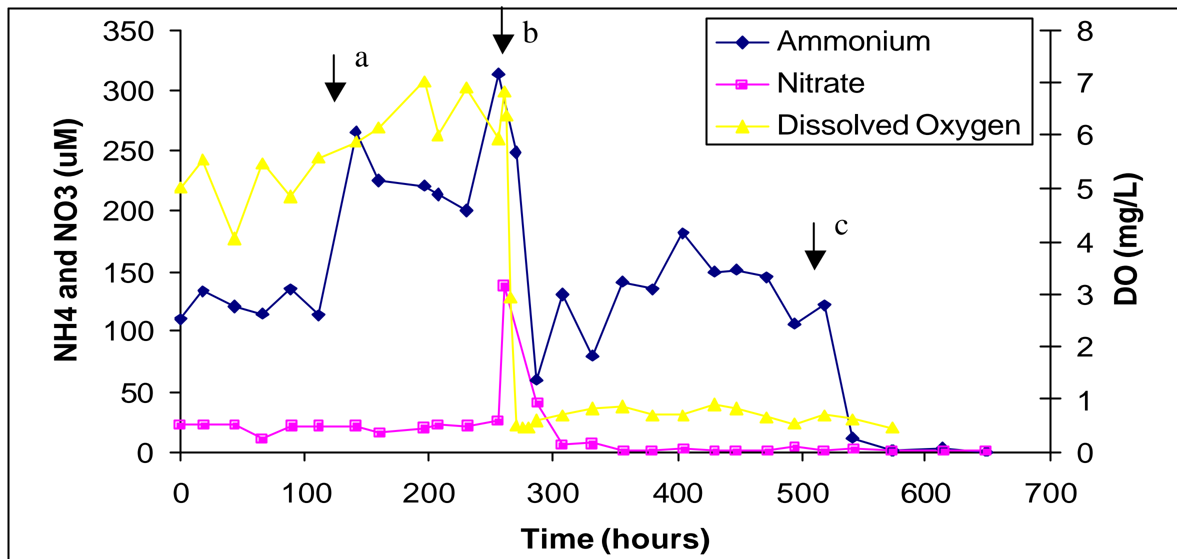

Figure 3 Ammonium, nitrate and dissolved oxygen concentrations in the full diversity microcosm. Arrow "a" represents the addition 50uM ammonium sulfate at 140 hours, arrow "b" represents the addition of 1mM dextrose and 100uM potassium nitrate at 260 hours and arrow "c" represents the addition of 3mM lactate at 520 hours.

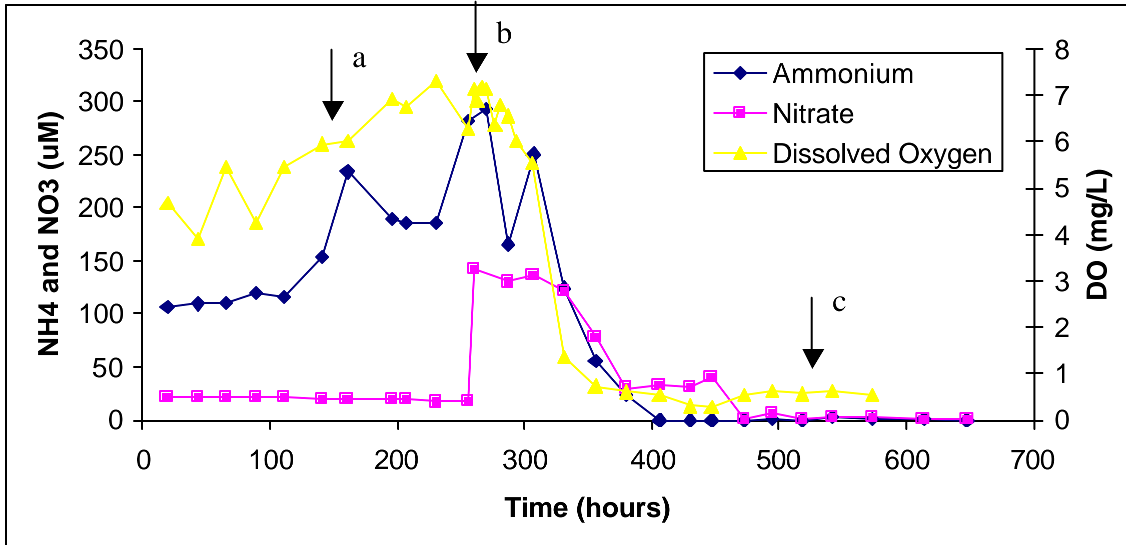

Figure 4 Ammonium, nitrate and dissolved oxygen concentrations in the low diversity microcosm. Arrow "a" represents the addition 50uM ammonium sulfate at 140 hours, arrow "b" represents the addition of 1mM dextrose at 260 hours and arrow "c" represents the addition of 3mM lactate at 520 hours.

The ammonium levels decreased in the full diversity microcosm until driven anoxic (Figure 3). Then, under anaerobic conditions, the ammonium concentrations varied little until the addition of lactate. Upon the addition of lactate (520 hours), the remaining 122µM ammonium was taken up within 24 hours. The ammonium levels in the low diversity microcosm did not vary until the system was driven anaerobic (Figure 4). Under anoxic conditions, the ammonium concentrations decreased rapidly to zero. In the full diversity microcosm, nitrate was entirely consumed within 40 hours of the addition of dextrose (Figure 3). The low diversity microcosm did not consume the entire available nitrate until 210 hours after the addition of dextrose (Figure 4).

DOC concentrations in the low and full diversity microcosms increased to 7-8mM upon the addition of 1mM dextrose ($C_6H_{12}O_6$) (Figure 5). Within 45 hours, the full diversity microcosm consumed 13 times more DOC than the low diversity microcosm. DOC concentrations slightly increased in both treatments upon the addition of acetate at 450 hours, and increased by about 9mM upon the addition of 3mM sodium lactate ($C_3H_50_3Na$).

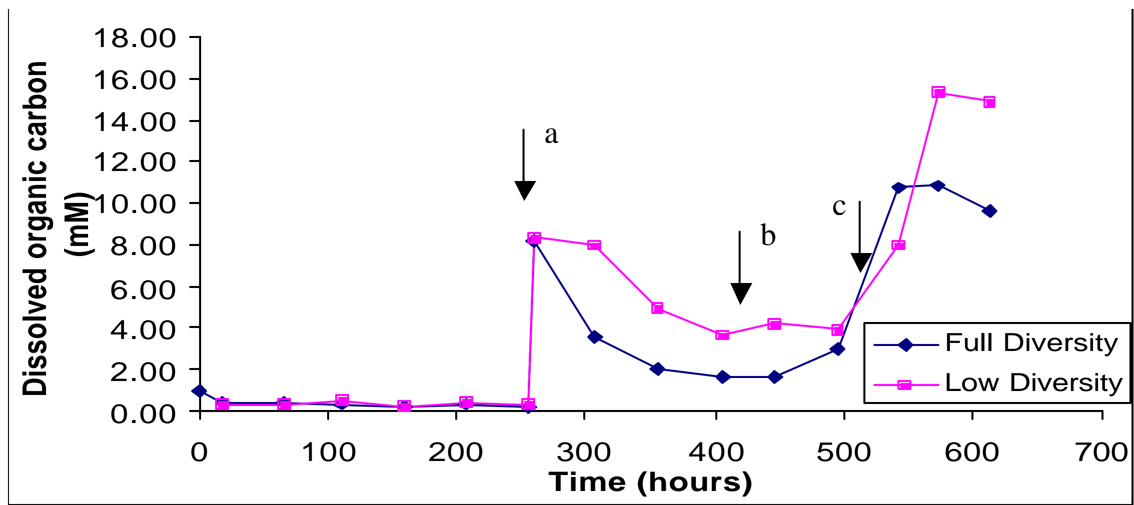

Figure 5 Dissolved organic carbon (DOC) concentrations in the full and low diversity microcosms. Arrow "a" represents the 1mM addition of dextrose at 260 hours, arrow "b" represents the 1mM addition of acetate at 450 hours and arrow "c" represents the 3mM addition of lactate at 520 hours.

Sulfate concentrations in the full and low diversity microcosms did not change throughout the experiment (Figure 6). Hydrogen sulfide test results in each carboy were negative.

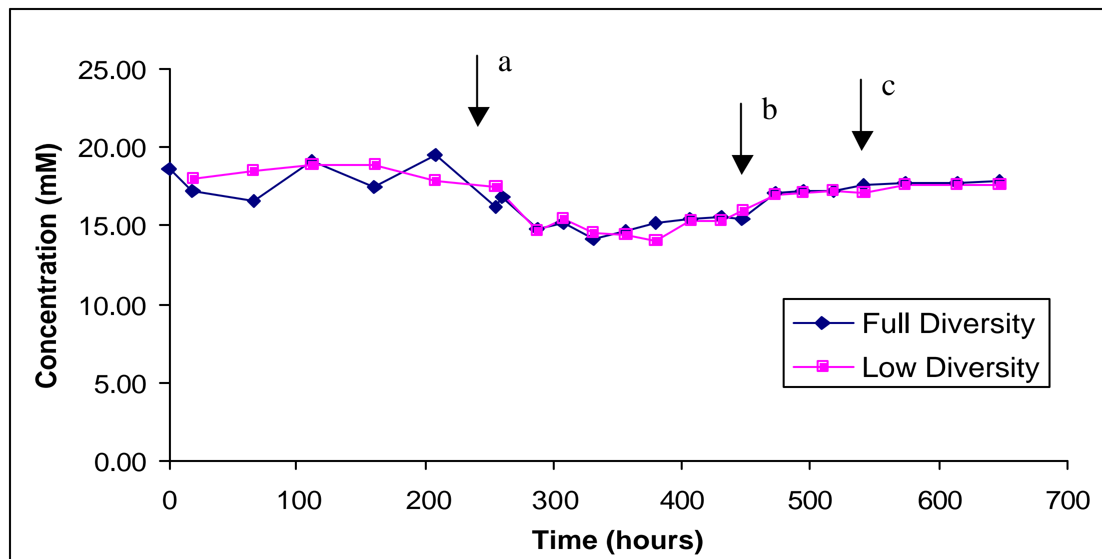

Figure 6 Sulfate concentrations in the full and low diversity microcosms. Arrow "a" represents the 1mM addition of dextrose at 260 hours, arrow "b" represents the 1mM addition of acetate at 450 hours and arrow "c" represents the 3mM addition of lactate at 520 hours.

Discussion

Upon the addition of dextrose, both microcosms showed dissolved organic carbon uptake (Figure 5), an increase in bacterial abundance (Figure 1) and a decrease in dissolved oxygen (Figure 2). These responses indicate that bacteria in both treatments

were able to utilize dextrose as a carbon-based energy source used for growth. The decrease in ammonium concentrations with bacterial growth suggests that bacteria in both microcosms were able to take up ammonium for protein synthesis. (Figure 3 and Figure 4). Nitrate was eventually used as an alternative electron acceptor in both microcosms under anaerobic conditions, indicating the presence of denitrifying bacteria in both treatments (Figure 3 and Figure 4). The preservation of denitrifying bacteria through the dilution was verified using PCR techniques (Levine and Charkoudian, 2001). Although the genetic diversity of the system decreased with dilution, the low diversity community was still diverse enough to utilize the same nutrient resources as the full diversity community, which was also observed by Ross (2000). This observation is consistent with the generalist/specialist concept, which predicts that generalists will be advantageous in unstable environments, whereas specialists flourish in relatively constant environments (Kitahara et al. 2000). Specialist microorganisms are capable of satisfying all niches with the most favorable energetic reactions in their environment, while generalists use sub-optimal metabolism to exploit a range of available organic compounds (Allsopp et al. 1995). Under high diversity conditions, generalists are out-competed by specialists. However, dilution selects for numerically abundant organisms, not just those that show superior growth in a given medium (Jackson et al. 1998; De Fede et al. 2000). Thus, generalist bacterial populations are able to thrive under low diversity conditions.

The gram stain test was used as a differential staining procedure to identify the unknown bacterium in each carboy as gram-positive or gram-negative. The lack of gram-positive bacteria in the low diversity microcosm suggests that the low diversity microcosm contains only gram-negative bacteria and that gram-positive bacteria were diluted out of the inoculum. PCR analysis indicated that ammonium oxidizers and universal Archeal functional bacteria were diluted out in the low diversity inoculum (Levine and Charkoudian 2001).

The amount of time each inoculum took to grow up to 10^7 bacteria per mL in the Reasnor Geldrich medium increased with each dilution most likely due to the decrease in bacterial abundance. The similar growth rates of the bacteria upon the inoculation of the carboys suggest that either the bacteria have the same growth productivity, interspecific

competition decreased with dilution (De Fede et al. 2000) or that the abundance of grazers decreased with dilution (Franklin et al. 2000). The slower rate of decrease in dissolved oxygen and DOC in the low diversity microcosm suggests a lag in response to the addition of dextrose (Figure 4 and Figure 7). This finding is consistent with general theories on the effects of diversity loss in microbial communities (Allsopp, D. et al 1995). The lag observed in the response of the low diversity microcosm to the perturbation suggests that the diversity of the microcosm affected the ability of the bacterial community to respond to the nutrient addition.

Although PCR techniques concluded that nitrifiers were present in the full diversity microcosm, ammonium concentrations did not decrease, nor did nitrate concentrations increase, during the aerobic acclimation period (Figure 3 and Figure 4). It is possible that the nitrifying bacteria were present in the microcosm but metabolically dormant. Many nitrifying bacteria, especially ammonium oxidizers, are inhibited by the presence of organic material (Madigan et al. 2000). The high concentration of labile carbon and other organic material may have inhibited the activity of the nitrifying bacteria present in the full diversity microcosm.

Sulfate is the next thermodynamically favorable electron acceptor after nitrate depletion in anaerobic environments. PCR techniques suggested the presence of sulfate-reducers in both the full and low diversity microcosm (Levine and Charkoudian 2001). However upon depletion of nitrate in the microcosms, neither decreases in sulfate concentrations nor increases in hydrogen sulfide concentrations were observed in the microcosms (Figure 6). This might also be explained by the presence of metabolically inactive organisms. Sulfate-reducers traditionally thrive in aquatic sediments, and it is possible that they were unable to survive suspended in the water microcosm. It is also possible that the time needed for the sulfate-reducing bacteria to adapt to the laboratory conditions resulted in a significant lag time in growth and activity.

The DOC concentrations throughout the experiment reflect a lag in response to the addition of different carbon substrates in the low diversity microcosm (Figure 5). Although neither microcosm showed a large response to the acetate addition, the full diversity microcosm responded quicker to the addition of dextrose and lactate, while the low diversity microcosm exhibited a delay in the DOC utilization.

According to bioenergetic models, which use basic principles of biochemistry, kinetic, and non-equilibrium thermodynamics to help understand the processing of DIN by bacteria, the full and low diversity microcosms exhibited a sub-optimal energetic metabolism under aerobic conditions (Vallino et al. 1996). The non-equilibrium thermodynamic model would predict nitrification, which did not occur during the acclimation period when the dissolved oxygen concentrations were high in the full and low diversity microcosms. Under aerobic conditions, ammonium is preferentially consumed over nitrate, which was observed in both systems upon the addition of dextrose and the ten-fold increase in bacterial abundance (Figure 3 and Figure 4).

Once oxygen levels were depleted and the microcosms were regarded anaerobic, the full diversity microcosm continued to exhibit optimal energetic metabolism, while the low diversity microcosm exhibited less than optimal use of resources. In the full diversity microcosm, ammonium consumption proceeded until anaerobic conditions prevailed, at which time nitrate consumption commenced. Under anaerobic conditions, the bioenergetic model also switches to preferential uptake of nitrate over ammonium (Vallino et al. 1996). The low diversity microcosm, however, continued to consume the ammonium under anaerobic conditions, and only upon depletion began the uptake of nitrate. The low diversity microcosm did not behave as predicted by the bioenergetic model based on growth rate optimalization. Although PCR techniques revealed the presence of denitrifers in both treatments, PCR analysis also suggested that dilution altered the community structure in the low diversity inoculum (Levine and Charkoudian, 2001). Perhaps organisms capable of switching to nitrate utilization over ammonium utilization as a response to the dextrose addition were diluted out of the community. The dilution appears to result in the inability of the low diversity community allocate resources in a manner that maximizes the growth rate of the community. This sub-optimal community structure of the low diversity microcosm inhibits the system's response to a perturbation.

Conclusion

The study suggests that a system can lose species diversity without losing functionality. However, the decrease in diversity creates a lag in the response of the

system to a perturbation. The loss of complementary redundancy due to a decrease in diversity also results in a system that exhibits sub-optimal energetic metabolism. The non-equilibrium thermodynamic model assumes that a community can take advantage of all possible niches. Low diversity communities, however, do not satisfy all possible niches, and thus may not operate in accordance to this model.

The chemistry of ecosystem processes is theoretically predictable by non-equilibrium thermodynamics for systems that satisfy all possible niches. However, it is difficult to simulate systems in which the biogeochemical processes transformations are linked to a low diversity population. The mechanisms of how microbial communities degrade following loss of diversity must be understood so that we can answer questions about the effects of the loss of microbial diversity on the sustainability of ecosystems.

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Appendix A

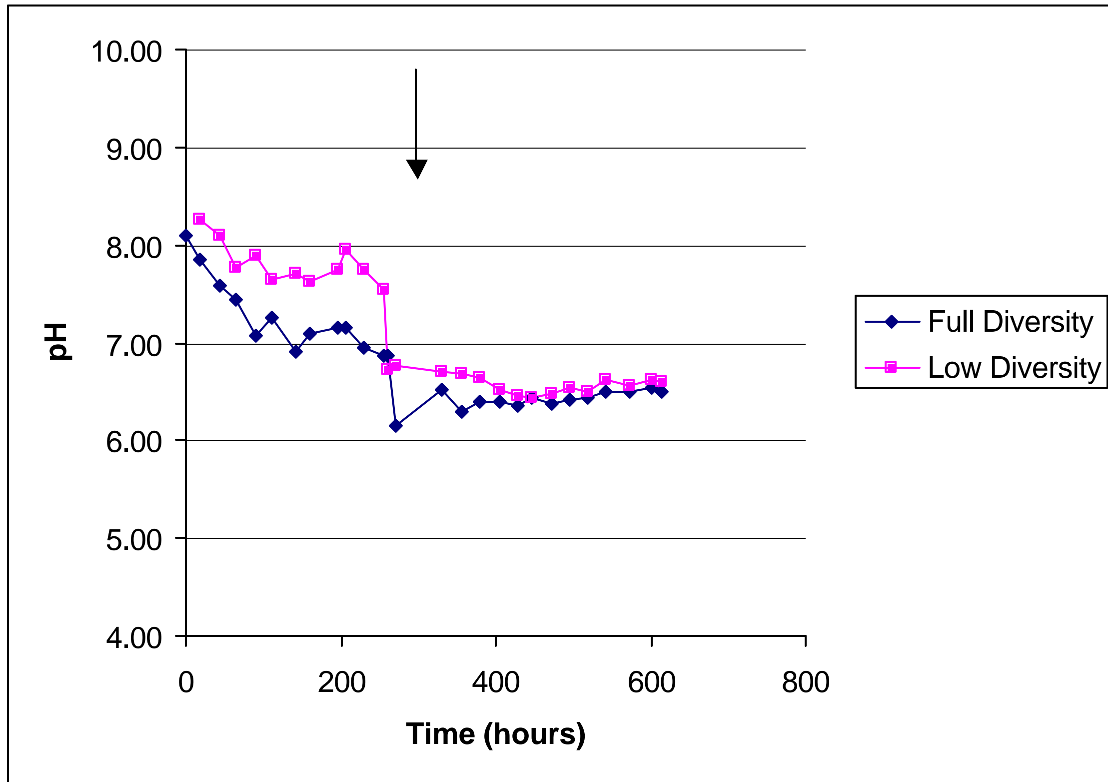

Figure A1 pH was buffered with 1mM sodium bicarbonate at 300 hours to maintain an optimal pH of 5-9. Arrow represents the addition of 5mM sodium bicarbonate buffer.

Appendix B

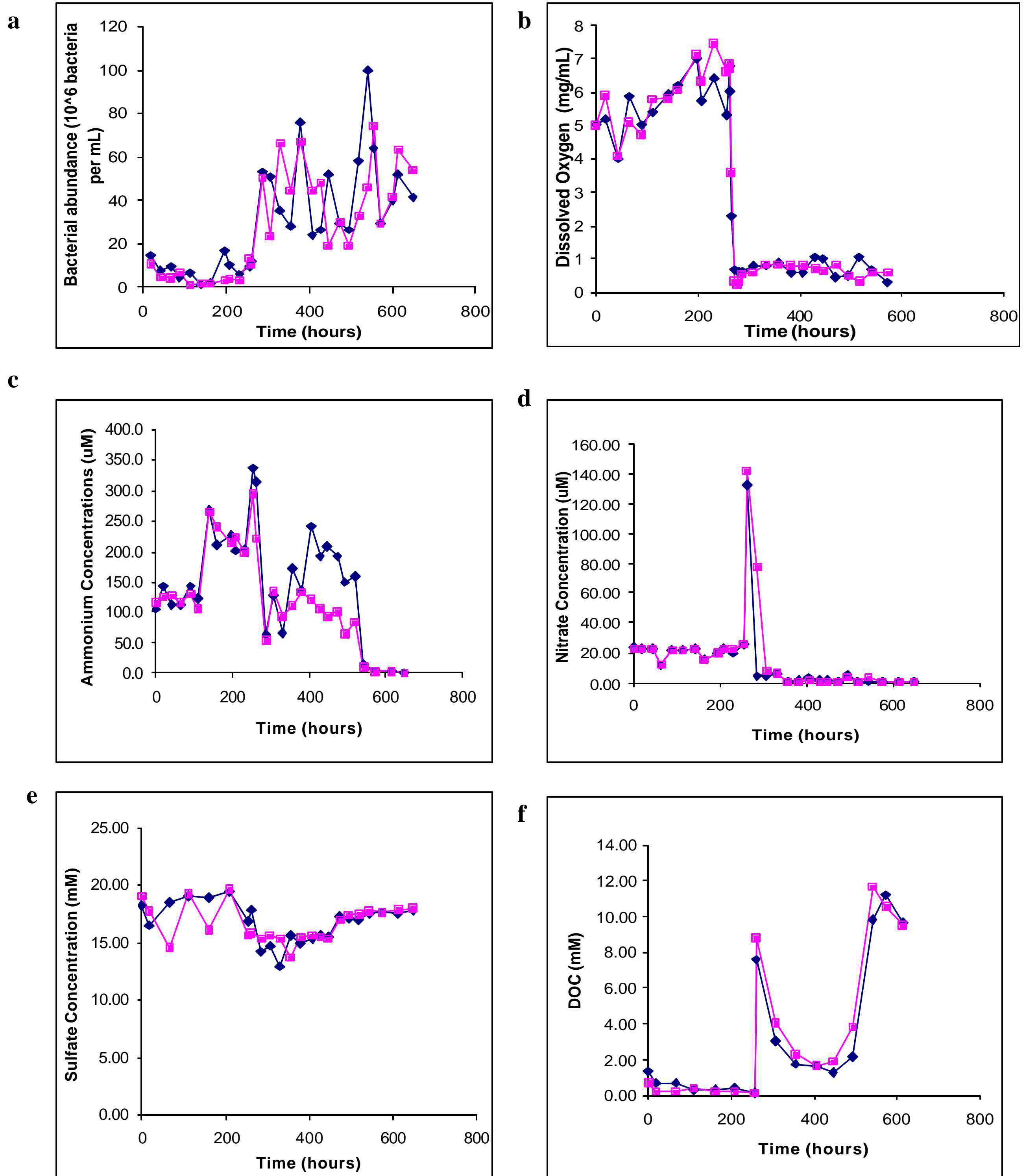

Figure B1 Full diversity replicates (F1 in blue, F2 in pink) show same trends for **a**) bacterial abundance **b**) dissolved oxygen concentrations **c**) ammonium concentrations **d**) nitrate concentrations **e**) sulfate concentrations **f**) dissolved organic carbon concentrations.

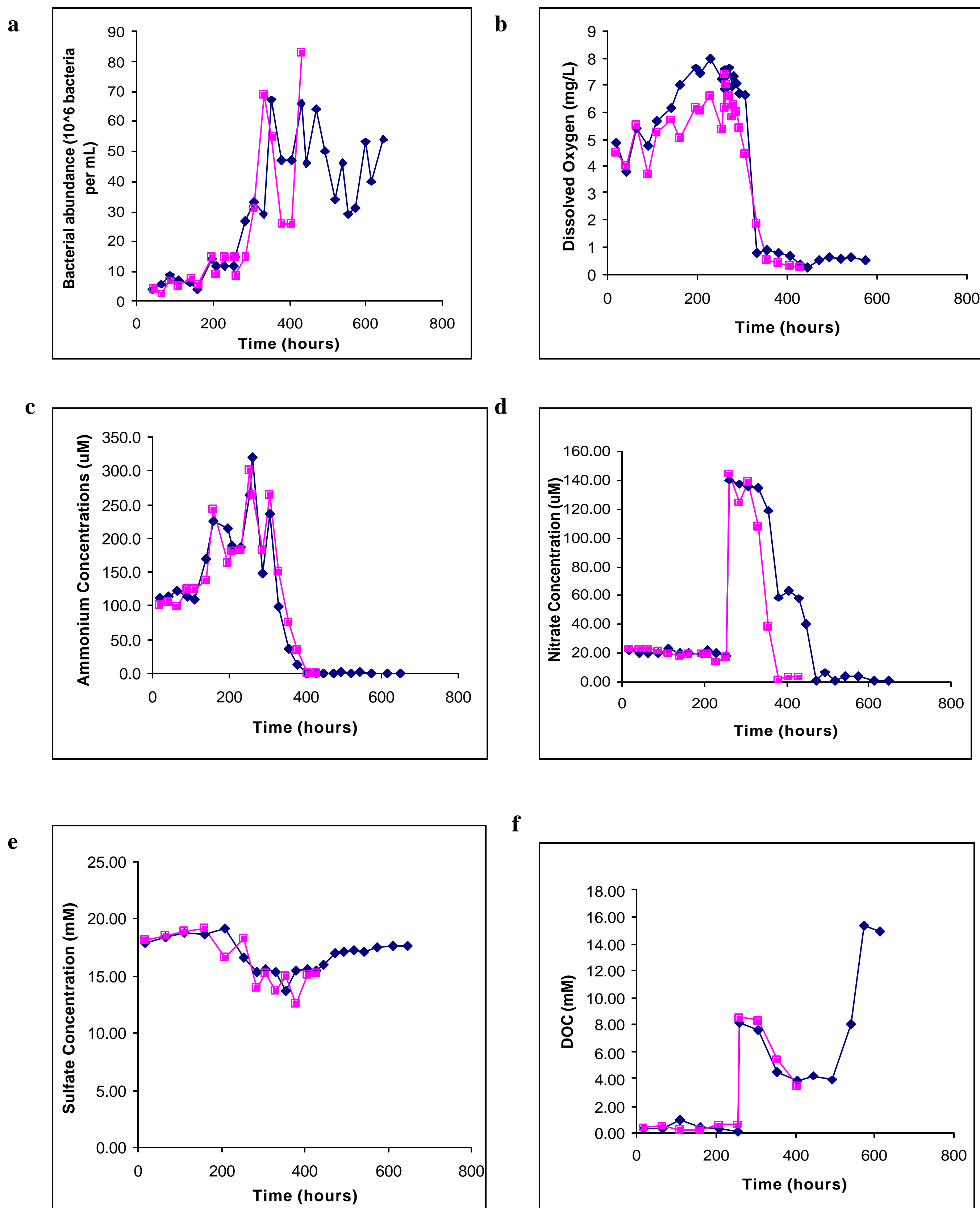

Figure B2 Low diversity replicates (L1 in blue, L2 in pink) show same trends for **a**) bacterial abundance **b**) dissolved oxygen concentrations **c**) ammonium concentrations **d**) nitrate concentrations **e**) sulfate concentrations **f**) dissolved organic carbon concentrations. Note that replicate L2 was lost at 430 hours due to a leak in the clamp.

Literature Cited

Allsopp, D., Colwell, R.R. and D.L. Hawksworth. 1995. Microbial diversity and ecosystem function. CAB International, Wallingford Oxon, UK.

Chapin, F.S. III., Walker, B.H., Hobbs, R.J. et al. 1997. Biotic control over the functioning of ecosystems. Science **277**: 500-504.

De Fede, K., Panaccione, D.G., and A. Sexston. 2000. Characterization of dilution enrichment cultures obtained from size-fractionated soil bacteria by BIOLOGR® community-level physiological profiles and restriction analysis of 16S rRNA genes. Soil Biology & Biochemistry **33**: 1555-1562.

Fieg, Y.S. and K.G. Porter. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanographic **25**(5): 943-948.

Franklin, R.B. et al. 2000. Impact of dilution on microbial community structure and functional potential: Comparison of numerical simulations and batch culture experiments. Applied and Environmental Microbiology **67**: 702-712

Garland, J.L., and R.M. Lehman. 1999. Dilution/extinction of community phenotypic characters to estimate relative structural diversity in mixed communities. FEMS Microbiology Ecology **30**: 333-343.

Griffith, B.S. et al. 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationships. Oikos **90**: 279-294.

Griffith, B.S. et al. 2001. An examination of the biodiversity-ecosystem function relationship in soil microbial communities. Soil Biology and Biochemistry **33**:1713-1723.

Gilboa-Garber, N. 1997. Direct spetrophometeric determinations of inorganic sulfide in biological materials in other complex mixtures. Analytical Biochemistry. **43**: 129-133.

Grime, J.P. 1997. Biodiversity and ecosystem function: the debate deepens. Science **277**: 1260-1261.

IUPAC Compendium of Chemical Terminology. 1997.

Jackson, J.R., Roden, E.E. and Perry F. Churchill. 1998. Changes in bacterial species composition in enrichment cultures with various dilutions of inoculum as monitored by denaturing gradient gel electrophoresis. Applied and Environmental Microbiology **64**(12): 5046-5048.

Jackson, Ethel N., and Lily Young. 2001. Learning and earning from diversity. Current Opinion in Microbiology **4**: 281-284.

Kitahara, M., Sei, K., and K. Fujii. 2000. Patterns in the structure of grassland butterfly communities along a gradient of human disturbance: further analysis based on the generalist/specialist concept. Popular Ecology **42**: 135-244.

Levine, U and Lou Caharkoudian. PCR analysis of microbial diversity in brackish water microcosms. Dec, 2001.

Madigan, M. T., Martinko, J.M. and Jack Parker. 2000. Brock biology of microorganisms. Prentice Hall, Inc, Upper Saddle River, New Jersey, USA.

Paerl, H.W. and J.L. Pinckney. 1996. A mini-review of microbial consortia: Their roles in aquatic production and biogeochemical cycling. Microbial Ecology **31**:225-247.

Peltzer, E.T., and Peter Brewer. Some practical aspects for measuring DOC- sampling artifacts and analytical problems with marine samples. Marine Chemistry. **41**: 243-252.

Pritchard, P.H. et al. 1995. The potential importance of biodiversity in environmental biotechnology applications: Bioremediation of PAH-contaminated soils and sediments. In: Microbial diversity and ecosystem function. Pp. 161-182. CAB International, Wallingford.

Ross, N., Villemur, R. E. Mercandella, et al. 2000. Assessment of changes in biodiversity when a community of ultramicrobacteria isolated from groundwater is stimulated to form a biofilm. Microbial Ecology. **42**:56-68.

Striekland, J. D. and T.R. Parsons. A practical handbook of seawater analysis. 1972. Ottawa Fisheries Research Boad of Canada 2nd Ed.

Vallino, J. J., Hopkinson, C.S., and John Hobbie. 1996. Modeling bacterial utilization of dissolved organic matter: Optimization replaced Monod growth kinetics. Limnol. Oceanog. **41**(8): 1591-1609.

Wardle, D.A., Bonner, K.L, Barker, G.M. et al. 1999. Plant removal in perennial grassland: vegetation dynamics, decomposers, soil biodiversity, and ecosystem properties. Ecol. Monogr. **69**: 535-568