

Response of estuarine phytoplankton and water column microbes to groundwater inputs down-gradient from a NITREXTM permeable reactive barrier.

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Abstract:

Anthropogenic nutrient loading to coastal estuaries has become a widespread problem particularly for the Cape Cod area where municipal sewage systems are lacking. Permeable reactive NITREX™ barriers, installed at the riparian zone of the Childs river and Waquoit bay, in Falmouth, Massachusetts, have been shown to effectively reduce this excess nitrogen input. This study attempted to look at the resulting effects when the barrier treated groundwater enters the estuary. The microcosm experiment measured the growth response of microscopic autotrophic and heterotrophic organisms residing in the water column. Due to the carbon component of the barrier material (wood chips) it was hypothesized that heterotrophic microbes would flourish in estuarine waters that meet with barrier treated groundwater. This expected result is in contrast to the excessive autotrophic growth, and harmful algal blooms, exhibited by non-barrier treated groundwater. Despite a groundwater sampling error, results showed that both autotrophic and heterotrophic growth are stimulated by high concentrations of nutrients, while concentrations remain stable where there are conservative nutrients available.

Introduction:

Excess nitrogen loading has become a large problem for coastal estuarine ecosystems in the Cape Cod area (Valiela, 2000). The excess nutrient loading over recent years has caused phytoplankton blooms to replace resident eelgrass populations and in turn leads to the hypoxia of higher trophic level organisms. One proposed solution to ameliorate this problem is the installation of permeable reactive NITREX™ barriers (PRB). These barriers are intended to be cost-effective de-nitrification systems that reduce the amount of inorganic nitrogen input into the estuary. This study looks at the resulting effects of these PRB's on the growth of downstream autotrophic and heterotrophic organisms as the "cleaned-up" groundwater enters the estuary.

It is well known that nitrogen pollution is problematic in estuaries. Likewise, it has been documented that these PRB's effectively remove the excess nitrate, before it enters estuarine waters (Moreau, S. 2005). Because these barriers are constructed with an organic carbon source (wood chips) it is expected that there will be dissolved organic carbon and dissolved organic nitrogen (DOC and DON) available to the microscopic organisms for consumption. This is in contrast to the high amounts of dissolved inorganic nitrogen (DIN) entering the estuary where there is no barrier treatment (Valiela, 2000).

Many nutrients in particular play an essential role in either autotrophic or heterotrophic growth. While phytoplankton, or autotrophs in general, typically utilize inorganic, mineralized forms of nitrogen the microscopic heterotrophs, or bacteria, will also utilize mineralized nitrogen where there is a high C:N ratio. On the other hand, if the dissolved organic matter is rich in organic nitrogen, and has a low C:N ratio, then bacteria will actually excrete excess ammonium, re-mineralizing the nitrogen themselves (Bratbak and Thingstad, 1985). At the same time bacteria may out-compete autotrophs for these nutrients because of their greater surface to volume ratio (Caron, et al., 1988). Regardless of population dynamics, heterotrophs require an organic carbon source for food and fuel, which they couple stoichiometrically with available nitrogen sources.

Therefore, in this microcosm study, we anticipated more of a growth response from the heterotrophic organisms downstream from the barrier treated groundwater where there is a rich carbon source, in contrast to the control sites, where there is typically an excessive autotrophic response. It was of additional question whether there would be a phytoplankton growth response as bacteria mineralize the organic carbon and nitrogen into inorganic forms making it available for primary production.

Materials and Methods:

Well point sampling kits were used to collect ground water at the riparian zone in four locations: downstream from the Waquoit barrier, Waquoit control, Childs river barrier, and Childs river control. Each water sample treatment was pre-filtered with a 47 μm filter and then filtered again with a .2 μm pore size cartridge filter (POLYCAP™) to remove, potentially, all microscopic organisms. Pre-filtered sea water was diluted 1:3 (30%) to make a concentration of 20 ppt – approximate to estuarine concentrations. It was then also filtered with the .2 μm cartridge.

850 mL of the seawater solution was then combined with 850 mL of groundwater according to treatment. 1700 mL total of combined water (sea water plus DI as a control, sea water plus Waquoit barrier water, sea water plus Waquoit control, sea water plus Childs barrier water, and sea water plus Childs control) were put into 2L acid washed plastic bottles with three replicates for each water treatment (15 microcosms total).

Actual estuary water was collected from both Waquoit Bay and Childs River locations. It was filtered with a 67 μm sieve to remove potential grazers and 200 mL was used to inoculate each of the solutions accordingly (Childs river inoculums went into the Childs river groundwater treatments, and likewise for Waquoit bay).

Microcosms were placed on a shaker table to keep samples mixed. The table was set up in a growth chamber with a controlled temperature of $\sim 18^\circ\text{C}$ and a twelve hour light cycle. Samples were taken for bacteria, chlorophyll, nitrate, and ammonium, dissolved organic carbon, and dissolved organic nitrogen concentrations over a sixteen day time course. Samples were taken at times of inoculation (time zero), 1, 2, 5, 8, 12, and 16.

Nitrates, ammoniums, DOC and TN were all filtered with a .7 μm GF/F, and either acidified and refrigerated, or frozen for later analysis (Solarzano, 1969) (Wood et al., 1967) (Moreau, 2005). Chlorophyll samples were run *in vivo*, at each time point, on a spectrophotometer and the Lorenzen extraction method was used at the final time point for a conversion equation (Lorenzen, 1967). For bacterial abundance measurements, 4 mL samples were placed into cryovials, then preserved with .284 μL of .2 μm filtered formalin, and frozen at -80°C for later analysis on a flowcytometer. A CTC stain was also used, at the final time point, to compare proportions of live bacterial assemblages to dead bacteria (Gasol and Aristegui, 2007).

Results:

Measured nutrient concentrations for Childs River and Waquoit Bay showed some unusual, interesting, and unexpected results. Initial nitrate concentrations were particularly high in both of the Childs river control and barrier samples at ~ 115 and ~ 92

μM respectively (figure 1). In consideration of the function and effectiveness of the barriers, the Waquoit bay nitrate measurements exhibited more reasonable concentrations as ~ 18 and $1 \mu\text{M}$ for the control and barrier samples respectively. The nitrate also remained constant over the 16-day time course in the Childs River samples and changed only modestly in the Waquoit samples.

Ammonium sample measurements resulted in concentrations that fluctuated considerably for all microcosms and in the Childs barrier assay in particular (figure 2). The concentrations were quite low however, and collectively, the dissolved inorganic nitrogen (DIN) closely resembled the pattern exhibited by the nitrate samples (figures 1 and 3).

Nitrogen that was measured as total nitrogen (TN) exhibited some change in concentration over the time course indicating that dissolved organic nitrogen (DON) was the most effected nutrient within all of the microcosms (figures 4 and 5). DON generally showed an increase at sample times 1 and 2 followed by a decrease in all treatments after the first few time points.

The chlorophyll concentrations follow behind the trends exhibited by the nitrogen, over the time course, but not always proportional to nitrogen concentrations (figure 6). There is an increase in chlorophyll after time point 2, for all treatments, where nitrogen decreases. However, in the Childs river microcosms the chlorophyll concentrations from the barrier treated water surpass the Childs river control treatment, while the control treatment exhibits greater nitrogen concentrations in all forms (figures 3, 4, and 5).

Bacterial abundance exhibited minor fluctuations relative to the chlorophyll time course (figure 7). Where there was high amount of nitrogen, followed then by a peak in chlorophyll, bacterial concentrations oscillated intermittently. There was a spike in bacterial abundance in the Childs river control treatment that did not correspond to the higher chlorophyll concentrations in the Childs barrier treatment, but did appear to follow to the higher concentrations of nitrogen for that treatment (figures 3, 4, and 5).

Dissolved organic carbon (DOC) was also measured but concentrations remained low and relatively stable throughout the time course (figure 8).

Discussion:

Previous PRB studies have indicated effective nitrate reduction to concentrations of approximately $5 \mu\text{M}$, or less, in the groundwater coming out of the barriers (Moreau, 2005). Therefore in this study the measured concentrations for the Childs river barrier treatment indicated a possible groundwater sampling error (figure 1). It is possible that during sampling the well point stake reached a depth beneath the barrier itself and obtained water that had not been de-nitrified. Childs river control water had high nitrate concentrations, as expected, for such a densely populated area. It is also unusual that the nitrate concentrations remain steady over the entire time-course and are not used by either autotrophs or heterotrophs. The nitrate values for Waquoit bay on the other hand exhibited the expected values consistent with previous studies and proper function of the barrier (figure 1) (Moreau, 2005).

Ammonium concentrations were apparently quite labile as they fluctuated between ~ 0 and $\sim 4 \mu\text{M}$ concentrations for all samples (figure 2). Due to the small

concentrations present of this nutrient, and its tendency to be favored by both bacteria and phytoplankton, it is not unusual that it would be utilized quickly and in turn be excreted over the time course (Pinckney, 2001). Species differences would also play a role in the nutrients that are utilized and further research might consider identifying autotrophs (or bacteria) present in the microcosms.

Both autotrophic and heterotrophic growth, that occurred in all treatments, exhibited a logical chronology in response to the nutrients available. Nutrients decreased at approximately time point 2, followed by an increase in chlorophyll at time point 5, while the bacterial abundance appeared to fluctuate in between these concentrations/populations (figures 1-8). However, in response to nutrient concentrations, an unexpected trend is exhibited by the chlorophyll concentrations in the Childs barrier treatment (where there is less nitrogen) and it surpasses the chlorophyll concentrations in the non-barrier treated water. This may also be due to the organisms that are present exhibiting different preferences and abilities in nutrient utilization, and the possibility that bacteria may be able to out compete autotrophs in some instances (Caron, et al. 1988).

Organic nitrogen and organic carbon do show corresponding trends with bacterial abundance in the Childs river control treatments (figures 5, 7 and 8). In organic forms, such as amino acids, bacteria will preferentially use the organic nitrogen as a high quality nutrient. All three of these measurements do show higher concentrations compared to the other treatments (Caron et al., 1988).

Interestingly, while there is a bacterial response to higher organic nutrients in the Childs river treatment, there is not much heterotrophic growth overall in all other treatments (figure 7). The exhibited outcome of the experiment does not actually show an increase in heterotrophic growth in the barrier treated water, as originally hypothesized, but rather that populations remained stable (figure 7). Chlorophyll responses were definitely reduced where inorganic nitrogen was reduced (figure 6). It appears that where nutrients are initially high in the Childs river treatments, both autotrophic and heterotrophic populations fluctuate quite a bit (figures 6 and 7). But where nutrients are conservative, both autotrophs and heterotrophs remain stable, which is itself a promising outcome.

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Figures and Tables:

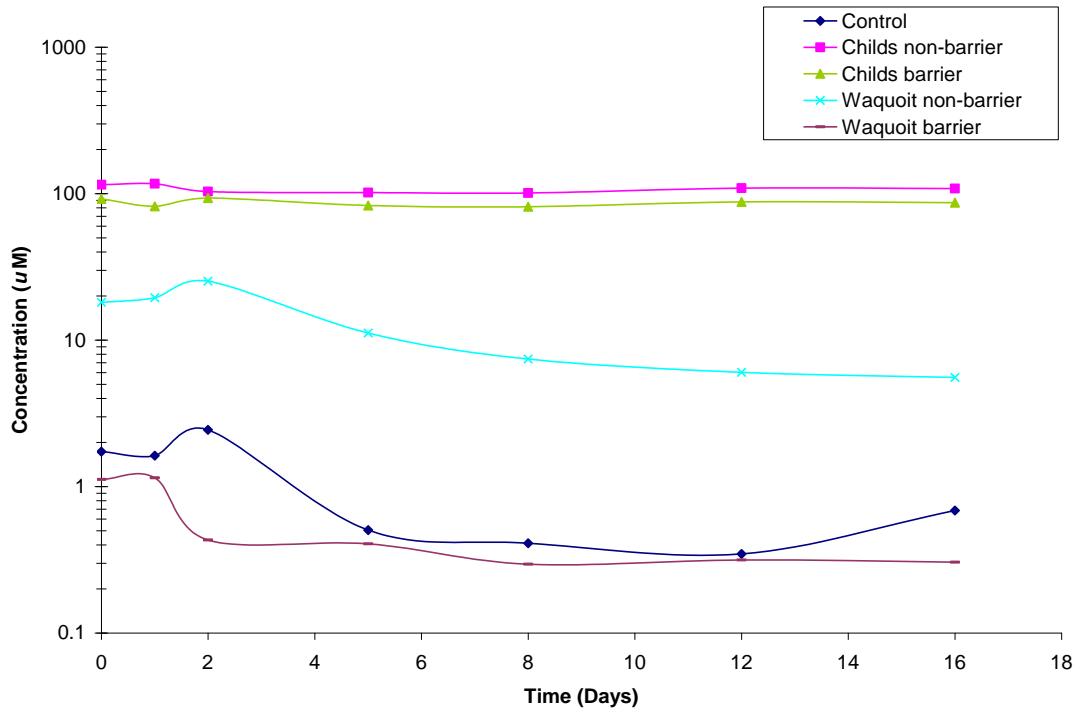


Figure 1: Nitrate concentrations measured at 7 time points, for 5 microcosm treatments, over a sixteen day time course. $.2 \mu\text{m}$ filtered groundwater, as the variable, was combined with $.2 \mu\text{m}$ filtered estuarine water, then inoculated with 200 mL of the respective estuarine water. Nitrate samples were analyzed on a Lachat Flow Injection Analyzer (Wood et al., 1967).

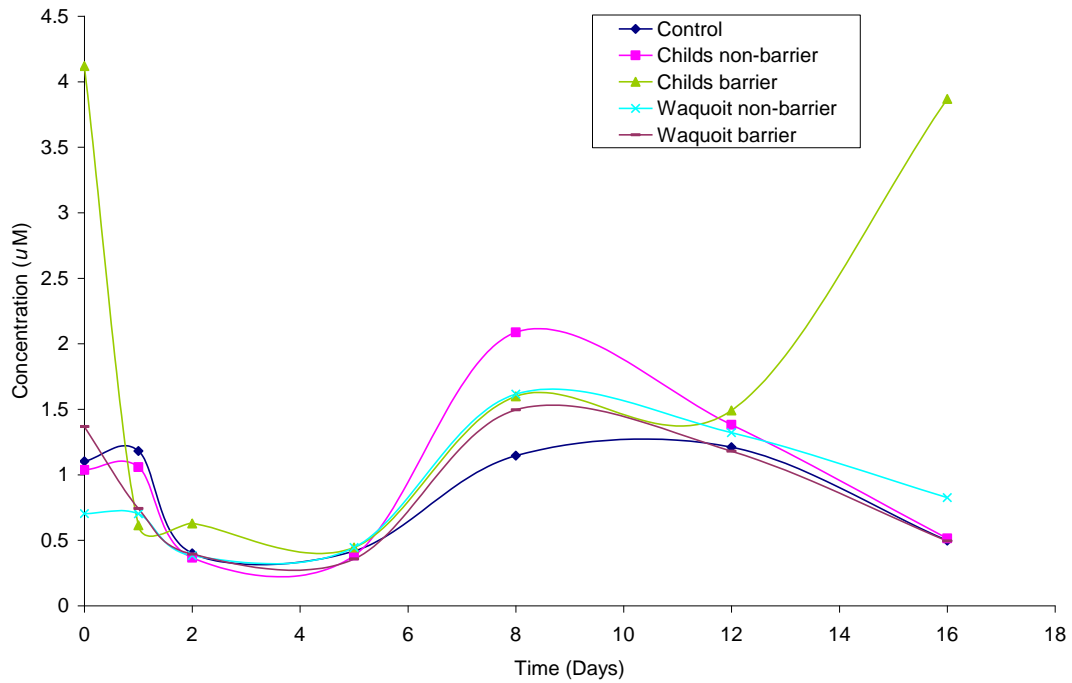
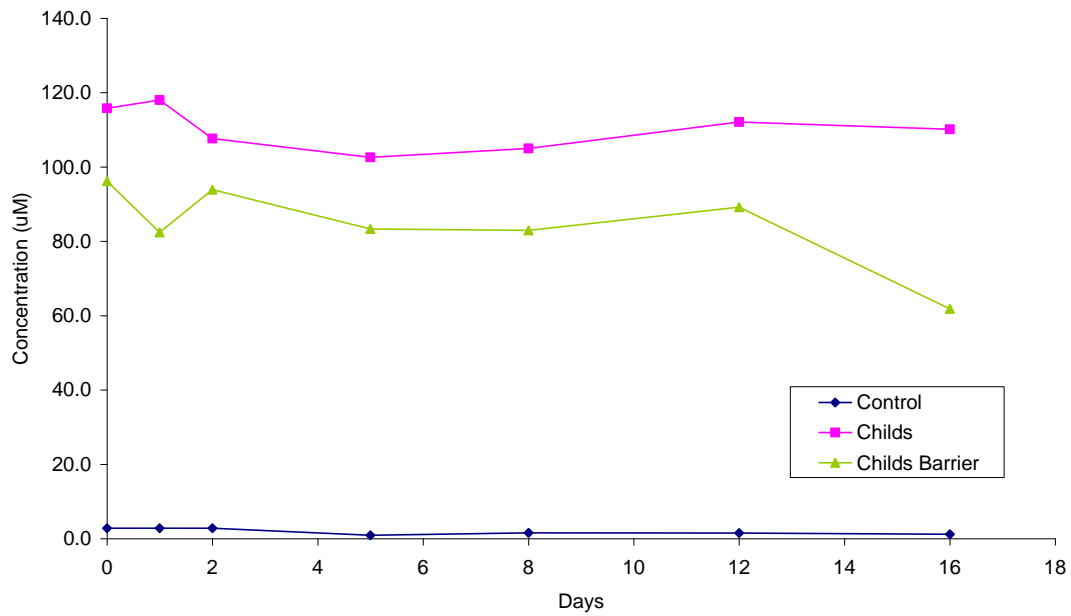
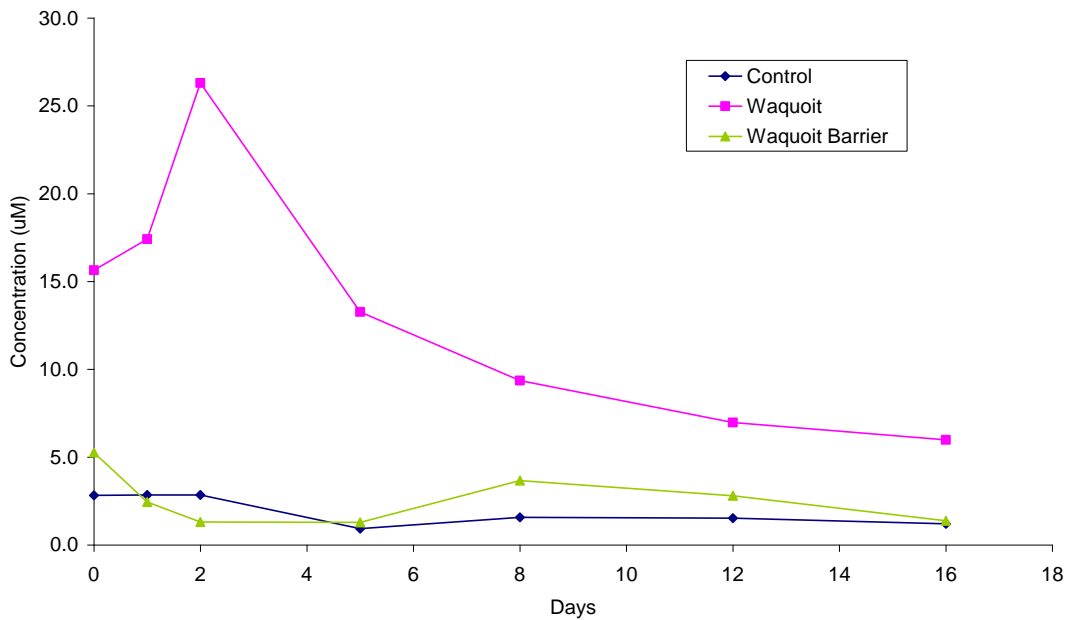


Figure 2: Ammonium concentrations measured at 7 time points, for 5 microcosm treatments, over a sixteen day time course. Treatments were the same as in figure 1. Ammonium samples were acidified with 10 μ L of 5N HCl and analyzed on a Shimadzu 1601 Spectrophotometer (Solarzano, L. 1969).

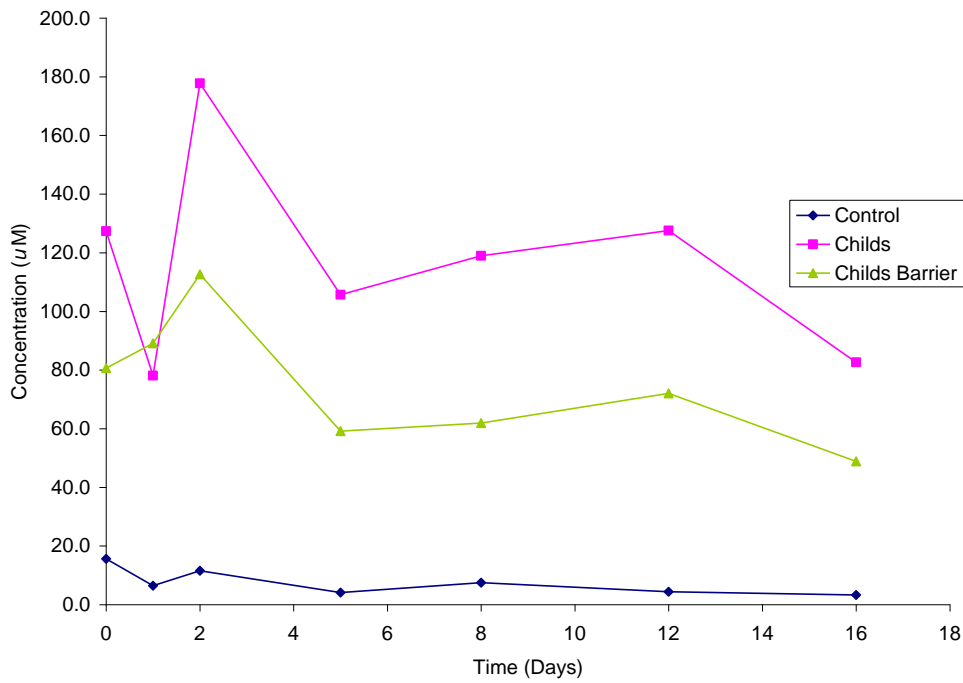


Childs River DIN

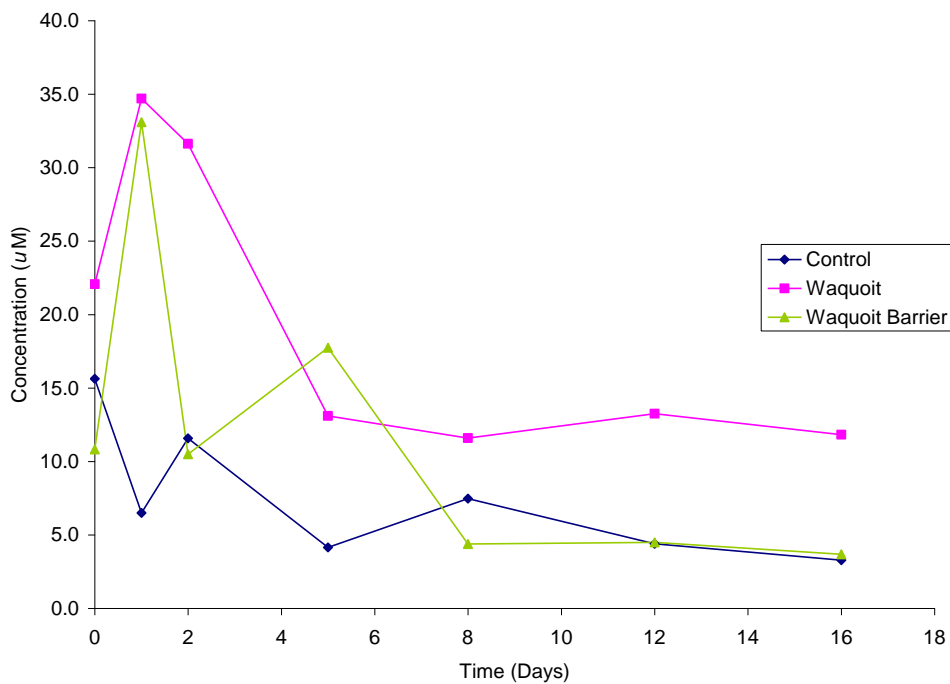


Waquoit Bay DIN

Figure 3: Dissolved inorganic nitrogen concentrations, for both Childs river and Waquoit bay, barrier groundwater and non-barrier groundwater microcosm treatments. DIN was determined as the sum of ammonium and nitrate, both measured at 7 time points, over a sixteen day time course (figures 1 and 2).

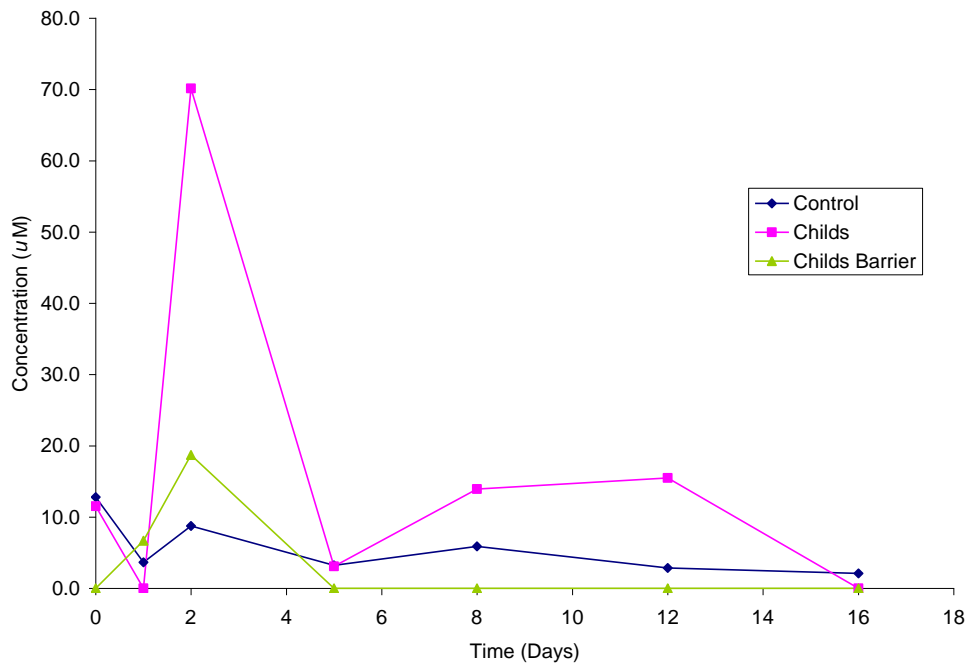


Childs River TN

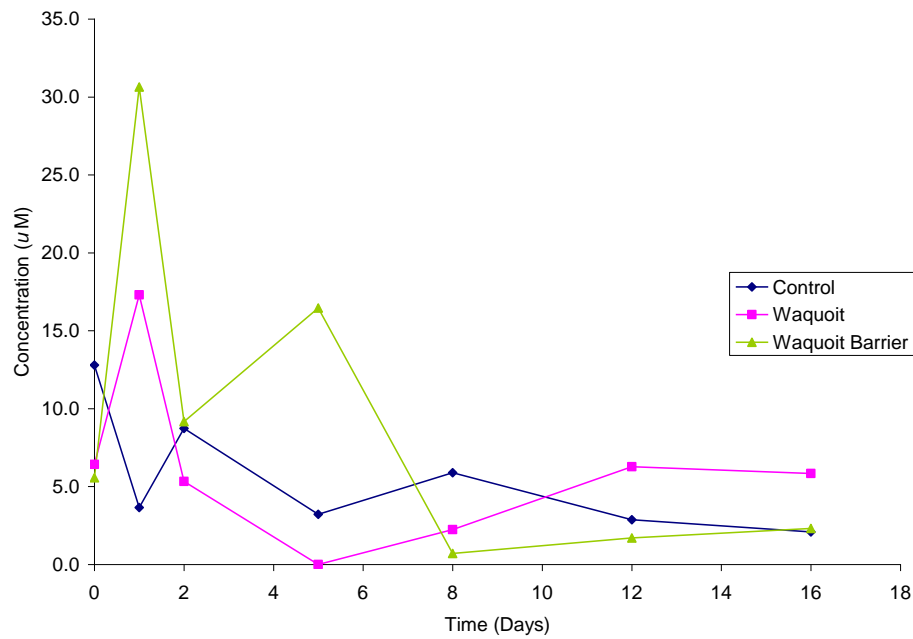


Waquoit Bay TN

Figure 4: Total nitrogen concentrations, for both Childs river and Waquoit bay, barrier groundwater and non-barrier groundwater microcosm treatments, measured on an HTCO machine manufactured by the Woods Hole Oceanographic Institute (Moreau, 2005).

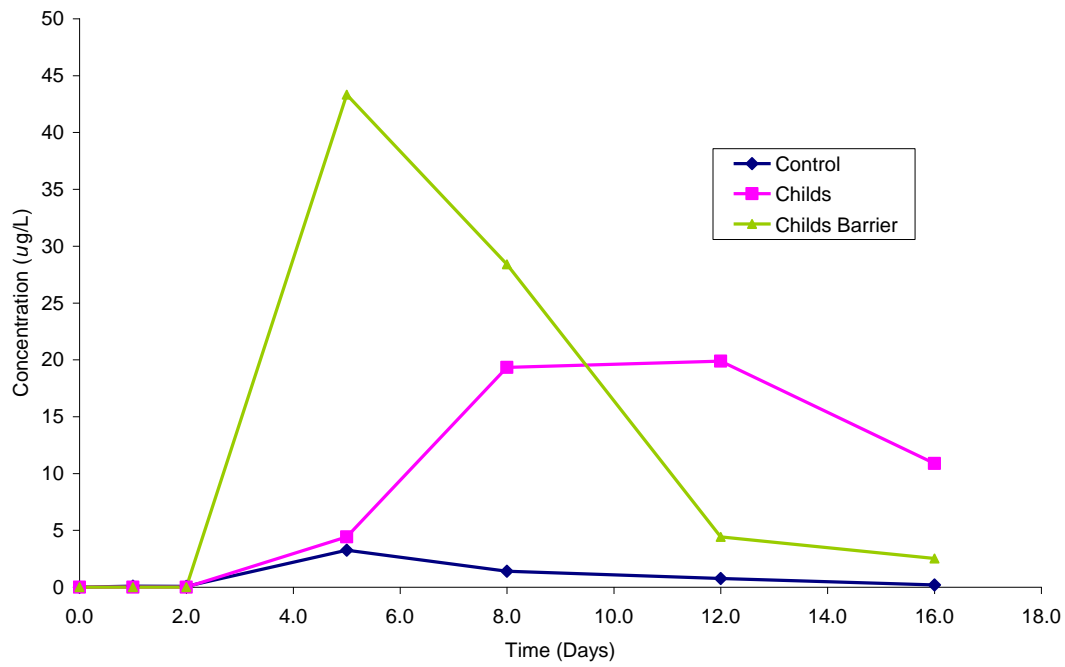


Childs River DON

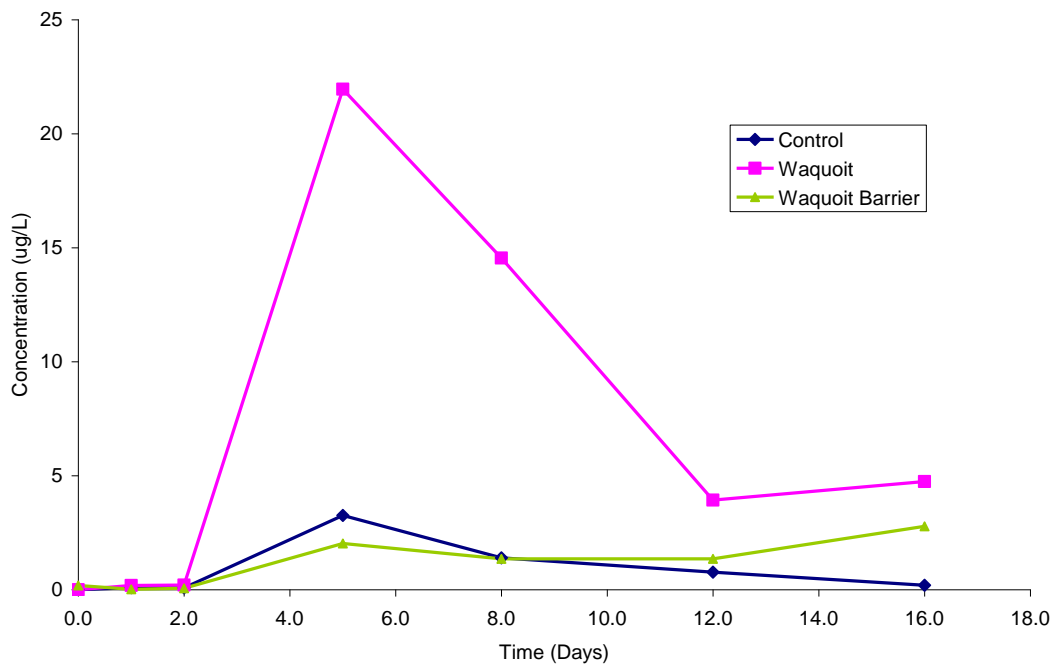


Waquoit Bay DON

Figure 5: Dissolved organic nitrogen concentrations, measured at 7 time points over a 16 day time course, for both Childs river and Waquoit bay barrier and non-barrier groundwater microcosm treatments. DON was determined as TN-DIN (figures 3 and 4).



Childs River Chlorophyll



Waquoit Bay Chlorophyll

Figure 6: Chlorophyll concentrations were measured using a flourometer at 7 time points, over a 16 day time course, for both Childs river and Waquoit bay, barrier and non-barrier groundwater treatments. Final time point samples were also measured, using the Lorenzen method (1967), to obtain a conversion equation for all other data points.

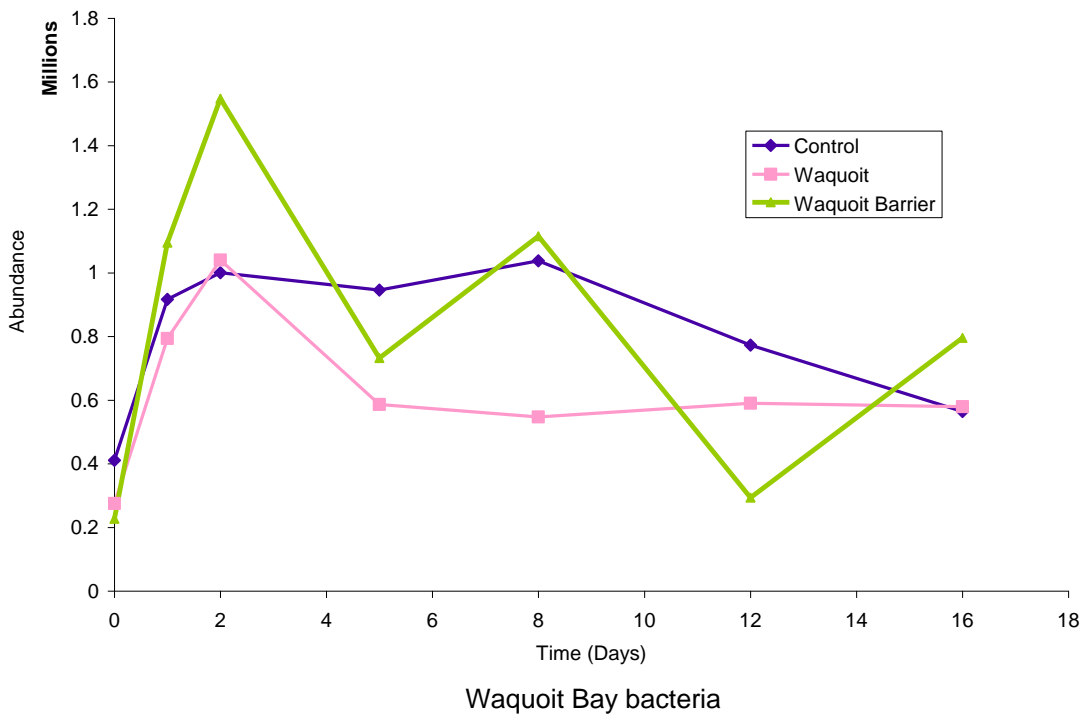
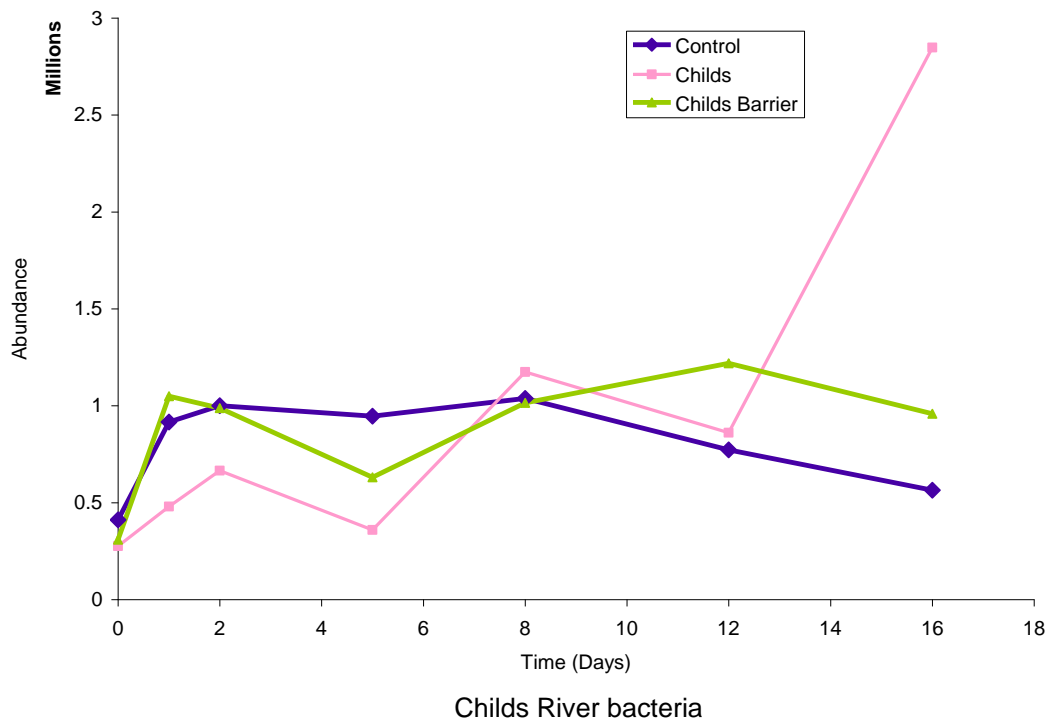


Figure 7: Bacterial abundance measured by flowcytometry at 7 time points, over a 16 day time course, for both Childs river and Waquoit bay, barrier and non-barrier groundwater treatments (Gasol and Giorgio, 2008).

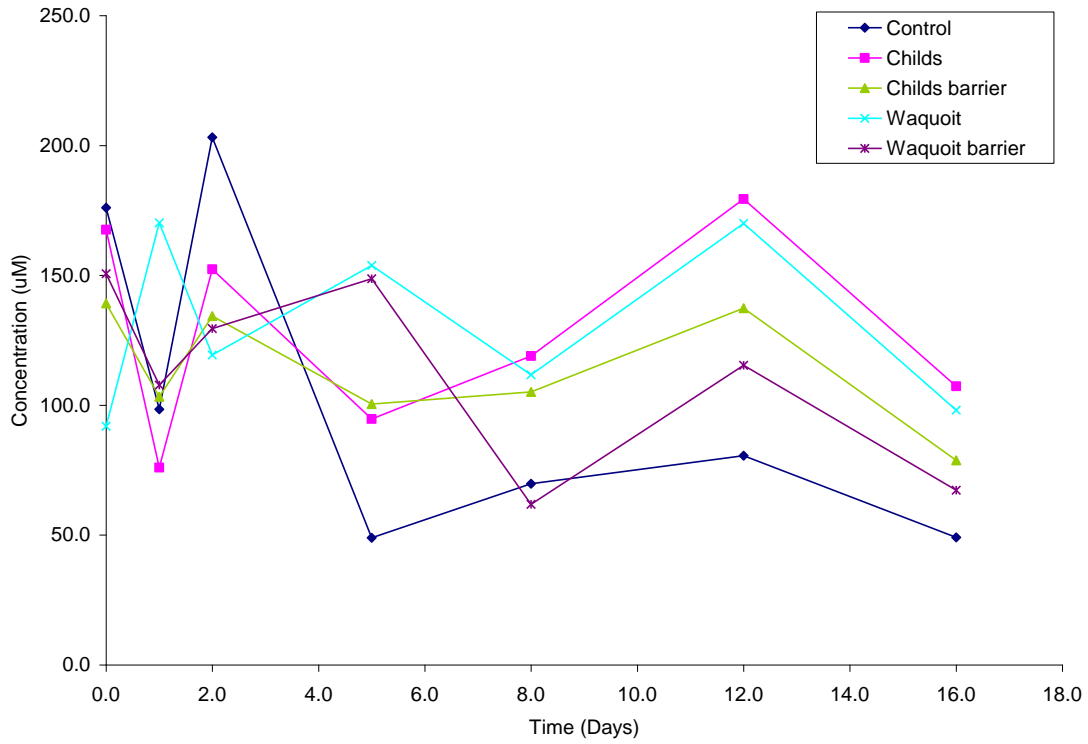


Figure 8: Dissolved organic carbon concentrations for all microcosm treatments, measured at 7 time points, over a sixteen-day time course. DOC was measured by an HTOCO machine manufactured by the Woods Hole Oceanographic Institute (Moreau, 2005).