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ORGANIC CARBON ABUNDANCE, DISTRIBUTION AND METABOLISM AT THE OYSTER, VIRGINIA STUDY SITE

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INTRODUCTION

Human society relies heavily on the relatively small pool of liquid freshwater available on land, of which the largest fraction (97%) occurs as ground water (Schlesinger, 1991 and references cited therein). Worldwide, there is concern about the quality of this freshwater supply as our reliance on subsurface aquifers increases in response to the demands of expanding world populations on dwindling surface water supplies. In addition to reductions in the volume of water stored in subsurface reservoirs, the quality of these water resources has also been compromised. Anthropogenic acitivities such as increased fertilizer and herbicide use, sewage runoff, waste disposal practices, and seepage from subsurface petroleum reservoirs have all contributed to declining water quality in aquifers.

In addition to concerns related to drinking water supplies, scientists have also begun to consider the ecological impacts of submarine ground water discharge on the coastal ocean as inorganic nutrients which could contribute to eutrophication are transported to nearshore and continental shelf waters (Capone and Bautista, 1985). The impact of ground water discharge on ecological processes may be more significant than previously thought given recent evidence which indicates that in regions such as the southeastern coast of the U.S., ground water discharge to the coastal ocean may be as large as 40% of the river water flux (Moore, 1996). The recognition of the potential magnitude of submarine ground water discharge and its effects on the coastal ocean (Moore, 1996; Bugna et al., 1996; Cable et al., 1996) has additional implications for geochemical and hydrologic budgets. Prior to these studies, riverine, hydrothermal and atmospheric sources of inorganic and organic constituents were thought to constitute the major fluxes to the oceans. Fluxes of inorganic and organic constituents delivered to the coastal ocean via ground water discharge must now be incorporated into global budgets.

Given the above considerations, it is important to understand the factors controlling the composition of ground water species. For several decades, it has been recognized that subsurface microbial communities are both abundant and diverse and can influence the

composition of inorganic and organic species (Lovley and Chapelle, 1995). Thus, understanding microbial respiration processes and the environmental factors controlling these processes is important to our ability to predict ground water chemistry. The availability of various electron acceptors determines how and with what efficiency microorganisms will utilize organic carbon compounds as substrates for metabolism. Microbially-mediated processes influence the Eh and pH of subsurface environments resulting in changes in the redox state of trace metal species and the dominant modes of microbial respiration. In recent years, there has been an increased focus on understanding the controls on these processes with the intent of using subsurface microorganims in remediation practices.

It is generally accepted that microbial activity in ground water systems is influenced by the abundance and composition of organic species associated with the dissolved and particulate phases (Chapelle, 1993). Organic constituents provide energy to microorganisms living in subsurface systems. Organic matter availability, however, is not controlled simply by its abundance. Recent studies have shown that organic matter availability is controlled both by the composition of organic species, as well as the physical matrix with which they are associated. For example, sorption of organic matter to surfaces (Keil et al., 1994) as well as within mesopores (Mayer, 1994) are possible mechanisms by which reactive organic matter is "protected" from microbial degradation. Likewise, the chemical composition of organic species (i.e., biological source, molecular weight, solubility, functional groups, aromatic vs. aliphatic character) may influence its usefulness to microorganisms. In ground water systems with little surface exchange, organic matter abundance and availability is largely controlled by the composition of the buried sediments. As a result, metabolic processes may become limited by the availability of reactive organic matter in systems with low levels of sedimentary organic matter.

This report describes a pilot study conducted at the DOE Subsurface Science Program's study site in Oyster, VA. The objective of this study was to examine whether

organic matter associated with the solid and dissolved phases was labile enough to support microbial activity. Organic matter availability was assessed in two ways: (1) by quantifying the amount and distribution of total organic carbon (TOC) associated with the solid phase and (2) laboratory experiments to examine the utilization of dissolved organic matter by measuring total microbial respiration. In addition to assessing total respiration, we specifically addressed organic matter respiration via denitrification. The focus on denitrification was due to the environmental field conditions at the study site (low concentrations of dissolved oxygen and high nitrate concentrations) suggesting that nitrate respiration would be a likely process for organic matter utilization.

METHODS

Characterization of Solid-Phase TOC

The DOE Subsurface Science Program's study site is located on the Eastern Shore of Virginia near Oyster (Fig. 1). Samples for determining the total organic carbon content of the solid-phase were collected from cores obtained from the nearby borrow pit (Fig. 1) during August, 1995. These cores provide a detailed grid of the study site, as well as coverage of the various facies present in the system. For this study, we subsampled two cores: UCA-2 (37°17.593 N, 75°55.50 W) and TOMO 5 (37°17.592N, 75°55.508 W). In addition, a small set of subsamples was collected from a third core, UCB-3 (37°17.595 N, 75°55.522 W).

Sediment samples were collected from multiple depths above and below the water table using solvent-cleaned spatulas. All samples were transferred to pre-combusted glass vials (450 °C for 5 hours) and stored frozen until analyzed. Following collection, the sediment samples were dried at 60 °C and ground with mortar and pestle. Subsamples were weighed into sample cups, treated with acid to remove inorganic carbon, and analysed on a Carlo Erba CHN Elemental Analyzer to obtain the weight percent TOC (Hedges and

Stern, 1979). At least two replicates of sediments collected from each depth were analyzed.

Laboratory Experiments to Assess Organic Matter Lability

Two amendment/incubation studies and organic carbon characterization were used to assess the effects of bacterial abundance, organic carbon, and nitrate supply on total community respiration, and nitrate respiration/denitrification in ground waters from Oyster, VA. Ground water from two wells located in the lower flow field of the DOE Subsurface Science Program's Study Site were studied (Fig. 2). The first experiment conducted during Spring 1996, investigated directly the influence of carbon supply and bacterial abundance on bacterial metabolism in high nitrate, moderately oxygenated ground water from well F3. A second experiment (Summer 1996) investigated the role of carbon and nitrate supply on bacterial metabolism in low nitrate anoxic ground water from well D1, as well as provided seasonal information on the role of carbon and nitrate limitation in ground water from well F3.

Amendment/Incubation Experiment #1 - Spring 1996

Field Collection

Three well volumes were purged from well F3 prior to sampling. After well purging, ground water was pumped directly into autoclaved, argon sparged carboys using a peristaltic pump. Water passed through a dual stopcock assembly with the displaced headspace vented through a water trap to minimize sample contact with the atmosphere. Dissolved oxygen and pH, were determined in the field using standard methods. A ground water subsample was reacted with ferrozine during sampling for later determination of soluble iron (Stookey 1970). Carboys were stored on ice during transport and incubations began within 24 hours of sample collection. Geochemical parameters at the time of sampling are provided in Table 1.

Table 1. Geochemical Parameters during April Experiment

Parameter	Well F3
Temperature (°C)	16
pH	5.99
Dissolved O_2 (mg L^{-1})	2.5-4.5
NO_3^- (μM)	191-193
NH_4^+ (μM)	1.2-1.8

Amendments and Incubation

Water (500 mls) was transferred anaerobically from the field collection carboy to autoclaved, argon-sparged 1000 ml erlenmeyer flasks. These incubation flasks were isolated from the atmosphere with a silicone stopper and dual stopcock assembly, and sample transfer was achieved by pressurizing the carboy reservoir with argon and venting flask headspace through a water trap (Fig. 3). Following sample transfer, the incubation flasks received one or a combination of the following amendments:

No addition (GW). Ground water only.

Nitrate (+N). Nitrate (KNO_3^-) was supplied at non-limiting concentrations (final incubation concentration greater than 200 μM-N).

Labile Carbon (+C). Labile carbon was supplied in non-limiting abundance (final incubation concentration approximately 3.0 mM-C). Carbon was supplied in excess of the electron requirement for carbon oxidation to CO_2 as well as conversion of NO_3----> N_2 via denitrification. Glucose and acetate in an electron equivalent ratio of 3:1 comprised the labile carbon substrate.

Bacteria (+B). Pure culture denitrifying bacteria (PL2W21) were added to the incubation flasks to acheive final incubation cell concentration of 10^6 cells ml^{-1}. PL2W21 was originally isolated from the Oyster site by Envirogen Inc. and identified as *Pseudomonas*

putida (Mary DeFlaun, pers. comm. 1996). Its denitrifying capability was confirmed by acetylene block assay (Knowles 1990) conducted in our lab in March 1996. A single PL2W21 colony was grown up in R2A broth (Mary DeFlaun pers. comm. 1996) 24 hours prior to the amendment/incubation experiment. Cells were washed in artificial ground water (Envirogen, Inc.), centrifuged, and resuspended twice prior to addition to the experimental flasks. Cell concentration of the culture was determined by optical density at 540 nm. The relationship between optical density and cell abundance had been previously determined by colony counts on R2A agar.

Acetylene (+A). Acetylene gas was added to a subset of the flasks in order to inhibit the reduction of N_2O to N_2 (Knowles, 1990). Acetylene was generated in a separate flask through the addition of calcium carbide to water. The acetylene filled headspace was removed with a syringe and injected into the aqueous phase in the incubation flasks so that the dissolved concentration was 15% by volume.

Sterile control (Kgw). Ground water was filter sterilized through a 0.2 μm in-line polyethersulfone (Supor) filter prior to incubation.

With the exception of the acetylene treatments, all flasks were sparged with argon following designated additions. Acetylene treatments were argon sparged prior to acetylene addition. Flasks were sealed and incubated in the dark at room temperature (20-25 °C) for up to 144 hours. Each of the treatments was performed in triplicate.

Table 2. Summary of Treatments for the April 1996 Experiment:

GW	GW+N	GW+N+C	GW+N+B	GW+N+B+C	Kgw
GW+A	GW+N+A	GW+N+C+A	GW+N+B+A	GW+N+B+C+A	Kgw+N

Sampling

To assess total carbon respiration and nitrate respiration/denitrification rates during incubation, flask headspace and aqueous fractions were sampled.

Headspace samples (10 ml) were withdrawn from the flask "headspace port" using a syringe fitted with a gas tight stopcock. Negative pressure imparted to the flask as a result of sampling was relieved by a simultaneous introduction of argon of equal volume through the aqueous port. One half of the headspace sample was analyzed for CO_2 using a Licor LI6252 flow through infrared CO_2 gas analyzer. The remaining headspace sample was analyzed for N_2O using a Shimadzu GC-8A gas chromatograph equipped with an electron capture detector and Poropak Q column. Headspace concentrations of CO_2 and N_2O were corrected for respective gas solubility according to Weiss (1974) and Weiss and Price (1980), respectively, yielding total CO_2 and N_2O evolution concentrations.

Aqueous samples were withdrawn from the "aqueous port" using a syringe while concurrently replacing the sample volume with argon through the "headspace port". Samples were analyzed for dissolved inorganic nitrogen (DIN; NO_3^-, NO_2^-, NH_4^+), dissolved organic carbon (DOC), bacterial abundance, and ferrous iron. DIN samples were filtered through a 0.2 µm Supor syringe filter, frozen and analyzed within one month. NO_2^- was determined by diazotization and $NO_2^- + NO_3^-$ by cadmium reduction in combination with diazotization (Alpkem 1992). NH_4^+ was determined spectrophotometrically according to the indophenol method (Solorzano 1969). In order to minimize contamination, DOC samples were withdrawn from the incubation flasks using new polypropylene (body and plunger) syringes, filtered through a 0.2 µm nylon syringe filter into ashed teflon capped glass vials. DOC samples were preserved with approximately 0.5 ml ultra pure 6N HCl and analyzed by high temperature catalytic oxidation on a Shimadzu TOC 5000 analyzer within three weeks of sampling. An

unfiltered aliquot of the aqueous fraction destined for bacterial abundance determination (5 mls) was fixed with 0.5 ml 0.3% gluteraldehyde, and stained with 4'6'-amidino-2-phenylindole (DAPI). Bacterial abundance was determined by direct count using epifluorescence microscopy (Turley 1993). Ferrous iron was determined on a 0.2 μm filtered subsample spetrophotometrically after complexation with ferrozine (Stookey 1970).

Upon termination of the incubation, the dissolved oxygen concentration in flasks was measured to insure that anaerobic conditions were maintained throughout the incubation. Flasks which leaked were removed from the data analysis pool. The pH was also measured at this time to determine contributions from the DIC pool to observed changes in the headspace CO_2 concentration.

Amendment/Incubation Experiment #2 - June 1996

A similar amendment/incubation experiment was performed in June 1996 using carbon, nitrogen , and acetylene additions (no bacterial amendments) to ground water collected from wells F3 and D1. Geochemical parameters at the study site are presented in Table 3 below. Experimental design followed a 7x2 factorial executed with an identical field protocol and incubation procedure as that described for the Spring 1996 experiment. Geochemical parameters at the time of sampling are presented in Table 3 and treatments for the Summer 1996 experiment are summarized in Table 4.

Table 3. Geochemical Parameters during June Experiment

Parameters	Well F3	Well D1
Temperature (°C)	17	17
pH	6.206	6.06
Dissolved O_2 (mg L^{-1})	1-2	<1
NO_3^- (μM)	211	< 1.0
NH_4^+ (μM)	4.84	283
Fe^{+2} (μM)	0	50

With the exception of quantifying bacterial abundance, the sampling protocol, chemical analyses, and data analyses were identical to that described above. Bacterial enumeration of selected subsamples of ground water (not incubated) from F3 and D1 were performed using DAPI-stained and Live/Dead-stained epifluorescence direct counts. DAPI staining was performed on 10 ml samples of water from both F3 and D1. Live/Dead stain was used on both 5 ml and 2ml samples from well D1, and a 5 ml sample from F3. Either 3 or 6 µl of stain was added to the sample in a filter chimney fitted with a 0.2 µm nucleopore filter stained with irgalan black. Samples were incubated in the dark for 15 minutes and the liquid evacuated. Approximately one hundred cells were counted on each grid.

Table 4. Treatment Used During June Experiment

GW	GW+C	GW+N	GW+N+C
GW+N+A	GW+N+C+A	Kgw	Kgw+N

Dissolved Organic Carbon Characterization - Fall 1996

Approximately 20 liters of ground water from wells F3 and D1 were collected as previously described and filtered through an in-line 0.2 µm Supor filter into ashed (450 °C) aluminum trays and frozen. Frozen aliquots were lyophilized to concentrate dissolved constituents by approximately 5 fold relative to ambient concentrations. Subsamples of the concentrate were analyzed to assess the aromatic content by measuring absorption of UV light at various wavelengths. Molar absorptivities have been shown to yield qualitative information about the degree of aromaticity, source, extent of humification and possibly molecular weight of organic matter (Chen et al., 1977; Chin et al., 1994; Traina et al., 1990). We used the ratio of absorbances 465 and 665 nm (E4/E6) which has been shown to vary across different types of humic materials while remaining independent of the concentration of humic materials, as well as absorbances at 272 nm and 280 nm, to assess

whether there were differences in the aromatic content of ground water collected from wells F3 and D1.

RESULTS AND DISCUSSION

Carbon Abundance and Characterization

Solid-Phase Total Organic Carbon

Solid-phase total organic carbon (TOC) contents ranged from 0.01-10.4 mg g^{-1} dry weight sediment in the TOMO-5 core and 0.01-3.37 mg g^{-1} dry weight sediment in the UCA-2 core. A small number of samples were also analyzed from the UCB-3 core and these ranged from 0.05-0.10 mg g^{-1} dry weight sediment. Results from this component of the project are reported in Table 5. Expressed on a weight percent basis, TOC values generally ranged from 0.01 to 0.3% in core UCA-2 and 0.01-0.2% in core TOMO-5. With the exception of one depth horizon (4.7 m) in the TOMO-5 core where TOC comprised about 1.0-1.4% of the sediment mass, values were in good agreement with previous work by Zachara and Smith (Field Experiments in Bacterial Transport, SSP, DOE) who found that organic carbon content ranged from 0.023-0.42% for six samples collected from this site. Further characterization of the solid-phase organic matter was not performed due to the low abundances. Unfortunately, cores from the lower flow field, the region from which ground water was collected for our incubation experiments, were unavailable to us.

Dissolved Organic Carbon

Dissolved organic carbon (DOC) concentrations were determined in ground water sampled from well F3 during April and wells D1 and F3 during June and November. Concentrations of DOC in well F3 ranged from 1.45 ± 0.09 mg L^{-1} in April, 1.55 ± 0.24 in June, and 2.26 ± 0.15 in November. At well D1, DOC concentrations were 3.4 ± 0.08 mg L^{-1} in June and 3.4 ± 1.2 in November. In addition, DOC collected during November 1996 was concentrated and its aromatic content examined qualitatively. Aromatic contents

were similar at D1 and F3 indicating that this parameter does not explain the between-site differences in the quality of organic matter suggested from our incubation experiments (see below). The aromaticity assay, however, indicates that aromatic content increased by about 40% following the addition of acid, suggesting that a substantial portion of the DOC at each site is probably bound to iron or other inorganic species. This observation suggests that the DOC concentrations reported above underestimate the true ambient concentrations of DOC at the study site.

Table 5. Total Organic Carbon Content of Borrow Pit Cores from Oyster.

TOMO-5			UCA-2			UCB-3		
Depth	Mean	Range	Depth	Mean	Range	Depth	Mean	Range
(m)	(mg g^{-1})		(m)	(mg g^{-1})		(m)	(mg g^{-1})	
1.56	0.07	0.00						
1.99	0.27	0.01						
2.41	0.34	0.01						
2.54	0.17	0.01						
2.76	0.72	0.01						
3.04	0.17	0.01						
3.44	0.15	0.02						
3.69	0.45	0.01						
3.97	0.24	0.02						
4.6	0.36	0.03	4.59	0.11	0.01			
4.7	10.0	1.87						
4.7	10.4	2.81						
4.9	0.23	0.02	5.04	0.20	0.01			
5.03	0.08	0.01	5.34	0.18	0.05			
5.2	0.19	0.03	5.66	0.13	0.04			
5.3	0.09	0.01	5.94	0.03	0.01			
5.5	0.02	0.00	6.58	0.02	0.01			
6.1	0.17	0.01	6.78	0.03	0.01			
6.35	2.06	0.19	7.08	0.04	0.01			
6.8	0.17	0.02	7.38	0.03	0.01			
6.9	0.14	0.09	7.67	1.12	0.01			
7.35	0.01	0.01	7.74	3.37	0.15			
7.77	0.14	0.00	7.81	0.10	0.02			
8.02	0.13	0.02	8.22	0.18	0.01			
8.5	0.01	0.01	8.39	0.23	0.02			
9.2	0.15	0.04				9.16	0.07	0.01
9.3	0.09	0.04						
9.5	0.21	0.06						
9.7	0.09	0.01				9.66	0.05	0.00
10.1	0.64	0.09				9.99	0.10	0.01
10.1	0.42	0.05						
10.23	0.12	0.02						

Incubation Experiment #1: April 1996

CO2 Production Rates

Total microbial metabolism was assessed by measuring CO_2 production over time in flasks containing groundwater collected from well F3 and amended as described above. Carbon and bacterial additions had the greatest effect in stimulating CO_2 production (Fig. 4) suggesting that labile carbon and bacteria were limiting at this site. Nitrogen additions also resulted in higher CO_2 production rates despite the fact that ambient nitrate concentrations were around 200 μM. In flasks without carbon additions, acetylene amendments had no effect. However, CO_2 production rates decreased when acetylene was added to flasks amended with carbon alone, as well as when carbon and bacteria combined.

N2O Production Rates

N_2O production by denitrification was used to assess potential rates of organic carbon metabolism by one group of microorganisms, the denitrifiers. High ambient nitrate concentrations at well F3 suggested that in the presence of reactive organic matter, denitrification should be the dominant metabolic pathway. N_2O production rates were characterized by three phases: (1) an initial increase in N_2O occurring between t_0 and t_{6hrs}, (2) a period with little N_2O production ($t_{6\ hrs}$ through $t_{20\ hrs}$ for bacterial treatments and $t_{40\ hrs}$ for treatments without bacterial additions), and (3) a secondary period of N_2O production in treatments with carbon additions beginning at $t_{20\ hrs}$ (+B) or $t_{40\ hrs}$ (without B) and persisting through the termination of the experiment (Fig. 5). As only one sample was collected after these secondary increases in N_2O production, it is unclear whether production increased immediately following the sampling at $t_{20\ hrs}$ (or $t_{40\ hrs}$) and leveled-off or whether rates of N_2O production were continuous for the duration of the experiment.

Similar to the CO_2 production rates, the highest N_2O production rates were observed in flasks amended with carbon or carbon and bacteria. These results suggest that denitrification is limited both by the availability of reactive carbon and the absence of an

active microbial community capable of denitrification. Low rates of N_2O production indicate that even when labile carbon and denitrifying bacteria were added to the flasks, only a small fraction of the CO_2 production resulted from denitrification. Addition of acetylene had little impact on N_2O production as one might expect if denitrification were responsible.

Dissolved Inorganic Nitrogen (DIN)

The concentrations of DIN species (nitrite, nitrate and ammonium) were monitored over the incubation experiments (Fig. 6). Nitrate was the dominant species, present in the groundwater at concentrations approximating 200 μM. Concentrations of nitrate were reduced in treatments amended with bacteria (GW+N+B and GW+N+B+C), with a more dramatic effect in the latter. Nitrate concentrations decreased to 10% of the initial concentrations over a 24 hours incubation period while nitrite concentrations concurrently increased from 0.14 μM to 305.8 μM (Fig. 6). At the termination of the incubations including bacteria, nitrogen and carbon amendments (+N+C+B), denitrification accounted for <1% of the nitrate decrease, while nitrate respiration accounted for ~84% of the decrease in nitrate. There is also the possibility that PL2W21 acted as a nitrate respirer, reducing nitrate to nitrite which then reacted abiotically to produce N_2O. Generally, however, such abiotic production requires low pH. Final pH values in our incubation flasks indicate that an abiotic mechanism for nitrate reduction is unlikely here. In addition, nitrate was reduced completely to nitrite, which was in turn reduced to N_2O, in a preliminary test with a pure culture of PL2W21

Summary: Experiment 1

Results from this preliminary experiment indicated that nitrate respiration (NO_3^- --> NO_2^-) and production of N_2O (either by nitrification or dentirification) were limited primarily by the availability of carbon and capable organisms. Carbon amendments

enhanced N_2O and CO_2 evolution, as well as nitrate disappearance and nitrite production. While the highest rates of nitrate respiration and denitrification were observed in carbon treatments also amended with nitrogen and nitrogen + bacteria, almost no enhancement of these reactions was detected in treatments that lacked the carbon addition. In treatments without added bacteria (+N+C and +N+C+A), N_2O production was observed only in the absence of added acetylene, suggesting the possibility that nitrifying rather than denitrifying bacteria were responsible. This would require some contamination by oxygen although N_2O production by nitrifiers is maximal under near anaerobic conditions. Since carbon also appeared to enhance N_2O production, the nitrifiers involved were most likely heterotrophic rather than autotrophic. Nitrate respiration accounted for 16% of the decrease in nitrate observed in these treatments.

Incubation Experiment #2: June 1996

CO2 Production Rates

Rates of total metabolism in water collected from well F3 and incubated as described above were comparable to those measured during April (1000 μmoles C L^{-1}). We did not detect any between-treatment differences in CO_2 production during this experiment (Fig. 9). In comparison, CO_2 production in ground water incubated from well D1 was about three-fold higher (Fig. 9) and the highest CO_2 production rates were measured in the flasks amended with carbon (2913 μmoles C L^{-1}). We did not find differences in carbon metabolism for the GW, GW+N, and GW+N+C amendments. These results suggest that in contrast to site F3, metabolism at site D1 is not limited by the availability of labile organic matter.

N2O Production Rates

N_2O production rates for carbon and nitrogen amended samples in June were similar to those found previously in April at well F3 (< 400 nmoles N L^{-1}; Fig. 10).

Again, denitrification comprised a small fraction of the total respiration. In contrast to the April experiment, acetylene additions resulted in increased rates of N_2O production, provided that nitrate and carbon were also added (GW+N+C+A), suggesting the existance in June of a higher abundance of denitrifiers in the natural assemblage of bacteria than in April. The increased population of denitrifiers probably reflects the decreased dissolved oxygen concentration in June compared to April.

Although DOC concentrations were higher at D1 than at F3 (3.7 vs. 1.5 mg l^{-1}), we also found evidence for carbon limitation. Rates of N_2O production at D1 were highest for ground water amended with both carbon and nitrogen (GW+N+C and GW+N+C+A). Acetylene addition to D1 resulted in increased production of N_2O implying that denitrification was responsible (GW+N vs. GW+N+A). Production of N_2O with addition of nitrogen and acetylene, but no carbon, was an order of magnitude greater in samples from D1 than F3 suggesting that both the quantity and quality of DOC were higher at well D1. A comparison of N_2O production in samples from both wells amended with nitrogen, carbon and acetylene showed approximately four-fold more N_2O production in samples from D1, suggesting a bacterial limitation in F3.

Bacterial Abundance

Bacteria present in well D1 during June were larger, more abundant, and morphologically distinct from those observed in well F3. Bacilli predominated in well D1 whereas tiny cocci, primarily attached to particles, were most common in F3. Bacterial abundance in well D1 was 10-100x higher than in well F3 (Table 6).

Table 6. Bacterial abundance in wells F3 and D1.

Date	F3	D1
10 Dec 96	7.1×10^8 cells ml^{-1}	9.6×10^9 cells ml^{-1} 1.5×10^{10} cells ml^{-1}
11 Dec 96	1.1×10^9 cells ml^{-1}	8.2×10^{10} cells ml^{-1}

Dissolved Inorganic Nitrogen (DIN)

Nitrate concentrations decreased in the incubations of ground water obtained from well F3 which had been amended with carbon (GW+N+C) while nitrite concentrations increased (Fig. 7). In these carbon-amended treatments of groundwater collected from F3, denitrification accounted for <10% of the nitrate decrease, while nitrate respiration to nitrite accounted for >62% of the decrease in nitrate. Unlike our April observations, acetylene additions increased N_2O production (GW+N+C vs. GW+N+C+A), indicating that denitrification was responsible for the nitrate reduced.

In contrast to well F3, the dominant DIN species at well D1 was ammonium at concentrations approximating 300 μM (Fig. 8). When nitrate was added to flasks containing groundwater collected from D1 and amended with carbon (GW+N+C), nitrate was respired to nitrite. There was no effect in flasks amended with N or C alone. Acetylene addition (GW+N+C+A) suppressed nitrate respiration to nitrite (Fig. 8). Similar to F3, nitrate respiration at D1 dominated over denitrification. Over the initial 72 hours of the experiments, denitrification accounted for <1% of the nitrate decrease in treatments amended with carbon and nitrogen. When these results are calculated over the experiment's duration (168 hours), 2% and 45% of the decreases in nitrate concentration were attributable to denitrification and nitrate respiration, respectively.

CONCLUSIONS

Our experiments indicate that nitrate is the dominant DIN species at F3 while ammonium is dominant at D1. Despite high levels (200 μM) of nitrate at F3, neither the addition of exogenous carbon nor the addition of bacteria (PL2W21 at 10^6 cells ml^{-1}) had a substantial effect on CO_2 production. During both experiments (April and June), acetylene reduced metabolism. During our April experiment, nitrate was almost completely reduced to nitrite in flasks amended with bacteria, during the initial 20 hours of the experiment before N_2O appeared. Acetylene had no effect on the production of N_2O suggesting that

nitrous oxide was the final product of denitrification under the conditions in the flask. In the absence of added bacteria, some N_2O was produced by nitrification and was only limited by carbon after the initial 40 hours. Nitrate reduction rates were higher in June than in April (incubations at room temperature) and were limited by carbon availability. Nitrite was the major product of nitrate reduction; N_2O was a minor product.

In comparison, CO_2 production was not enhanced by the addition of carbon nor nitrate to ground water collected from well D1. Although bacteria did not limit denitrification rates in ground water collected from D1, denitrification still accounted for only a small fraction (<1%) of the nitrate decrease in treatments amended with carbon and nitrogen. Nitrate additions resulted in nitrate reduction to nitrite. Carbon additions enhanced nitrate reduction.

Results from this study suggest that even under environmental conditions, seemingly conducive to NO_3^- respiration, natural attenuation of ground water NO_3^- comprises a small fraction of the total microbial respiration in the lower flow field of the Oyster, VA site. Although environmental conditions (oxygen and nitrate concentrations) are appropriate for denitrification at site F3, the site lacks the appropriate microbial community and is carbon limited. In contrast, the absence of nitrate at site D1 precludes the dominance of metabolism by denitrifying organisms. With respect to overall microbial respiration, site F3 is carbon limited while site D1 does not appear to be. However, despite sufficient quantities of organic carbon at site D1, the process of denitrification was limited by the quality of that carbon.

We caution, however, that our experiments were conducted using ground water alone and subsequent experiments which assess the role of ground water and sediments from the same sites may provide very different insights. Observations made during this pilot study indicate that particulate microbial biomass is probably greater than found in the dissolved phase. Thus, any follow-up to this study should consider the role of microorganisms attached to the solid-phase.

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FIGURE CAPTIONS

Fig. 1. Location of the DOE Subsurface Science Program's study site on the Eastern Shore of Virginia. Note the location of the experimental plot and borrow pit.

Fig. 2. Locations of the monitoring wells and flow field wells at the experimental site. The lower flow field has three sets of flow cells (D,E, and F). Wells D1 and F3 were sampled for this study.

Fig. 3. Schematic of water transfer from the field collection carboy to autoclaved, argon-sparged 1000 ml erlenmeyer flasks. Incubation flasks were isolated from the atmosphere with a silicone stopper and dual stopcock assembly, and sample transfer was achieved by pressurizing the carboy reservoir with argon and venting flask headspace through a water trap

Fig. 4. Respiration, measured as CO_2 production, at well F3 during the April, 1996 experiment. Data points in this and subsequent figures represent the mean of three samples; error bars are the standard error.

Fig. 5. Denitrification, measured as N_2O production, at well F3 during the April experiment.

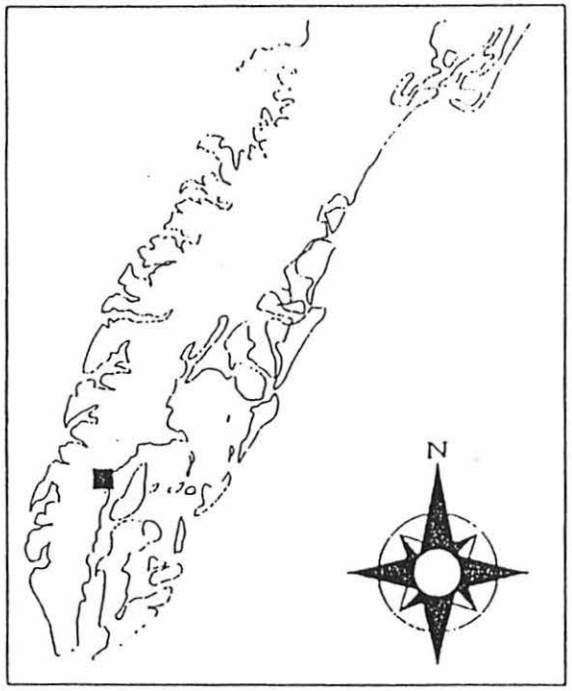
Fig. 6. Concentrations of dissolved inorganic nitrogen species (nitrite, nitrate and ammonium) at well F3 during the April experiment.

Fig. 7. Concentrations of dissolved inorganic nitrogen species (nitrite, nitrate and ammonium) at well F3 during the June experiment.

Fig. 8. Concentrations of dissolved inorganic nitrogen species (nitrite, nitrate and ammonium) at well D1 during the June experiment.

Fig. 9. Comparison of respiration rates in water incubations from wells F3 and D1 measured during the June experiment.

Fig. 10. Comparison of denitrification rates in water incubations from wells F3 and D1 during the June experiment.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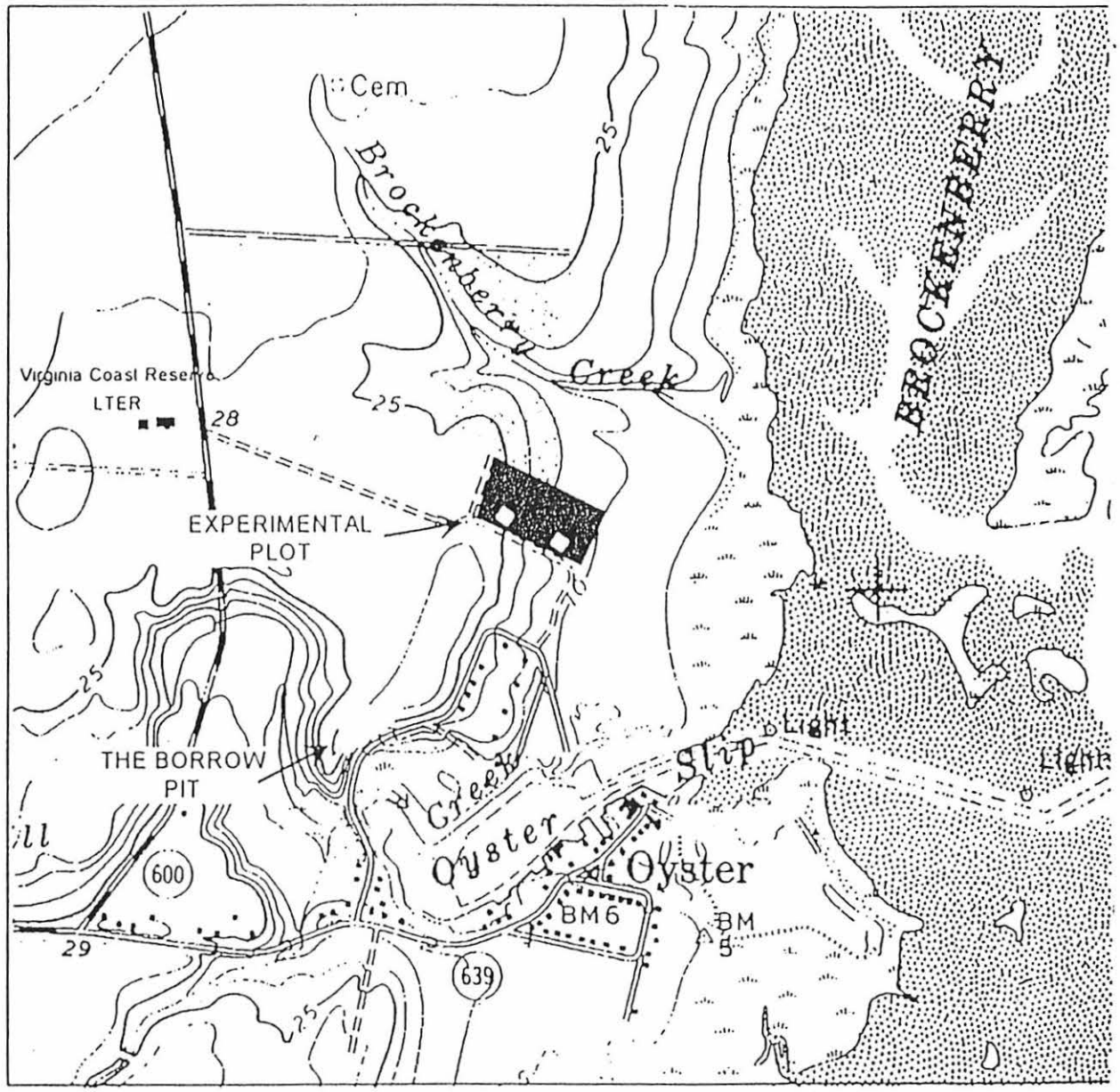

Cem
Brockenberry Creek
BROCKENBERRY
Virginia Coast Reserve
LTER
28
25
EXPERIMENTAL
PLOT
THE BORROW
PIT
Oyster
Oyster
Slip
Light
Light
600
639
BM6
BM
5
29

Fig. 1

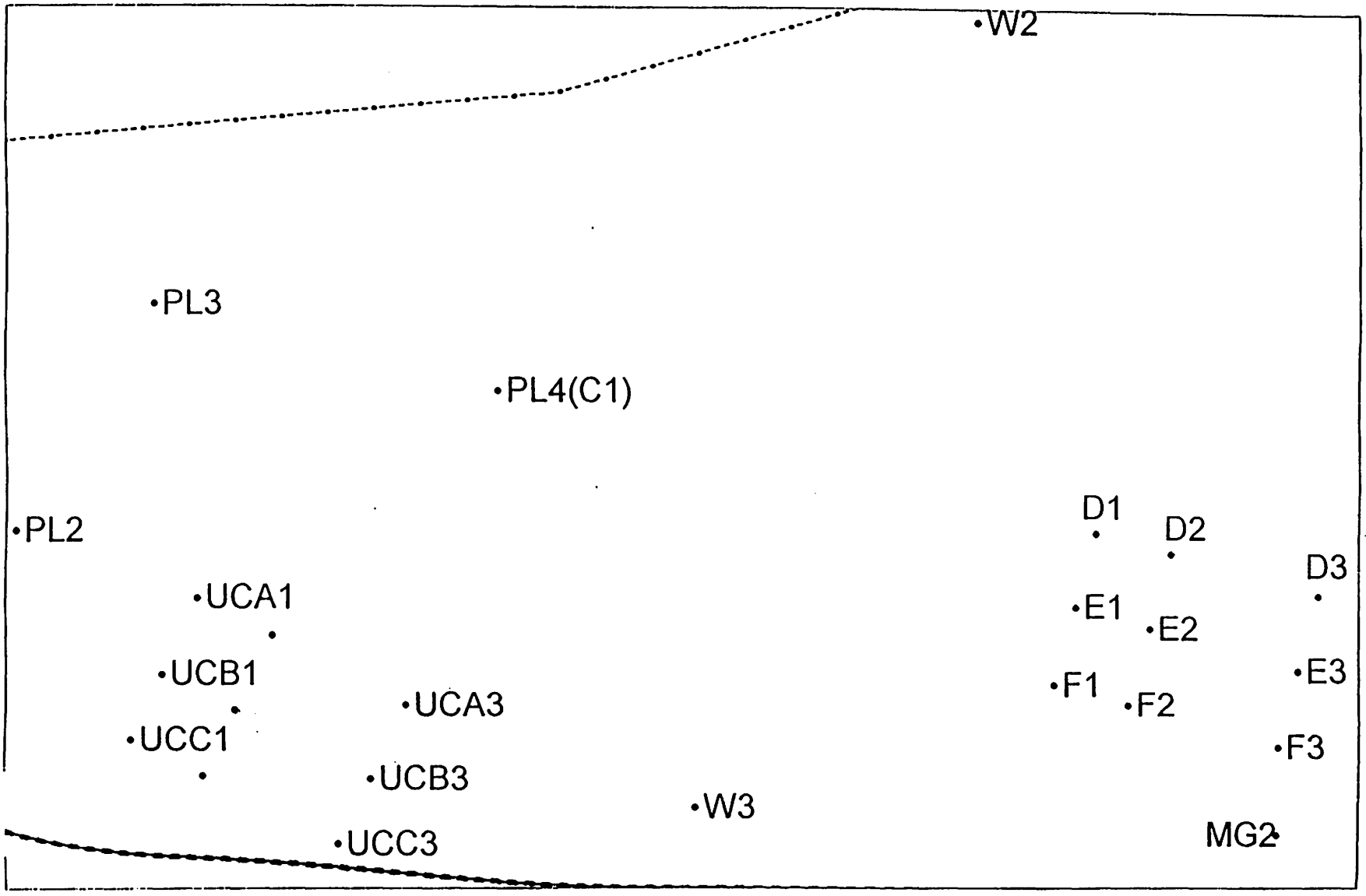

W2
PL3
PL4(C1)
PL2
D1
D2
D3
UCA1
E1
E2
UCB1
E3
F1
F2
UCA3
UCC1
F3
UCB3
W3
UCC3
MG2

Fig. 2

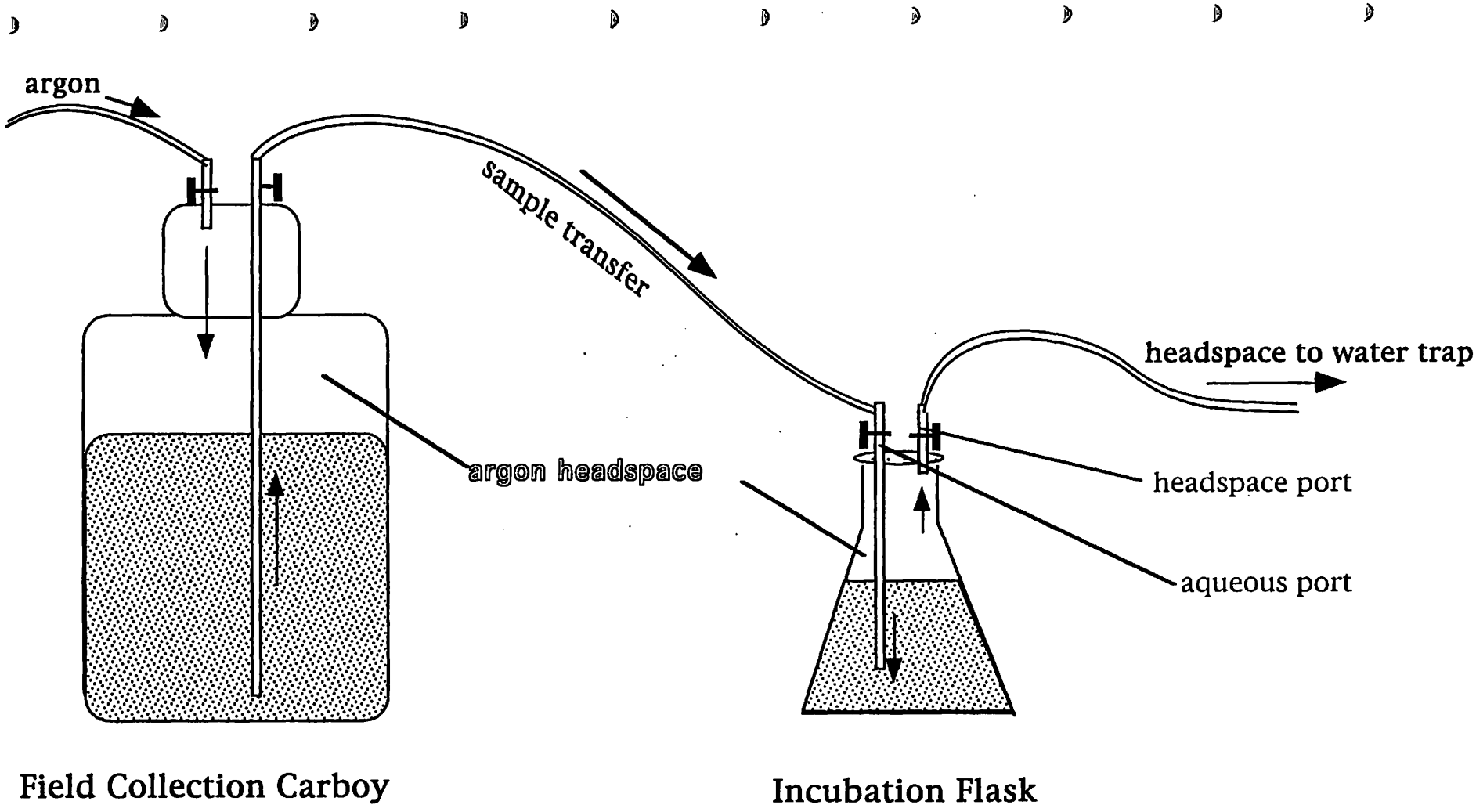
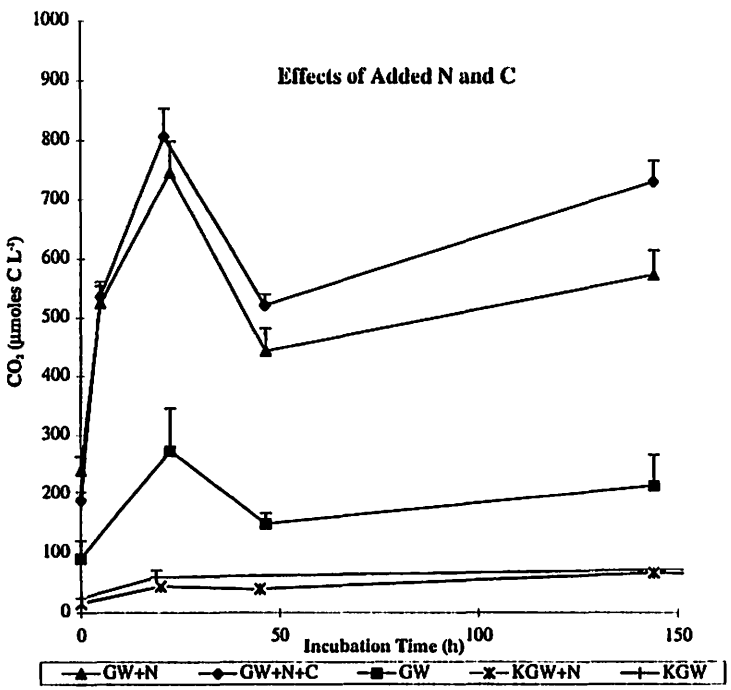
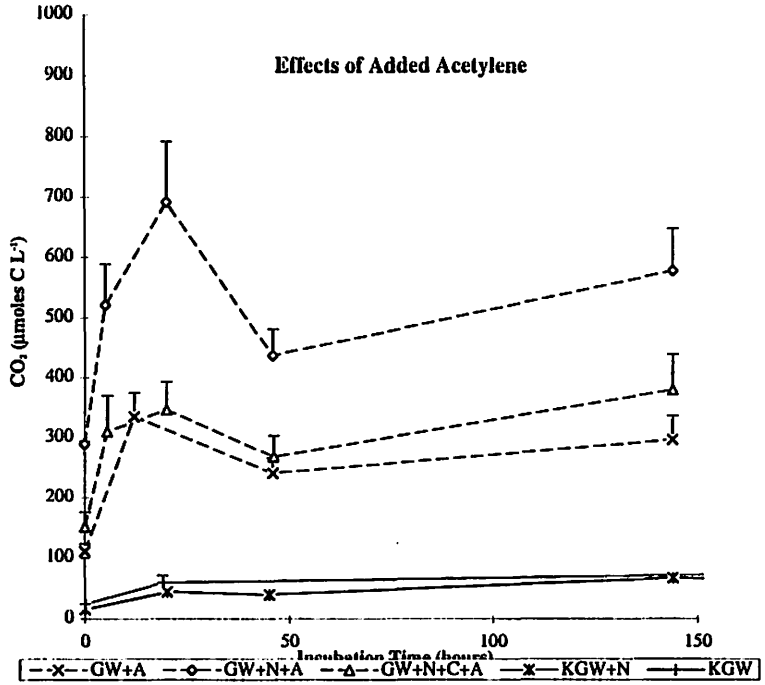


Figure 3. Sample Transfer Scheme

Figure 4. Respiration, Well F3, April 1996





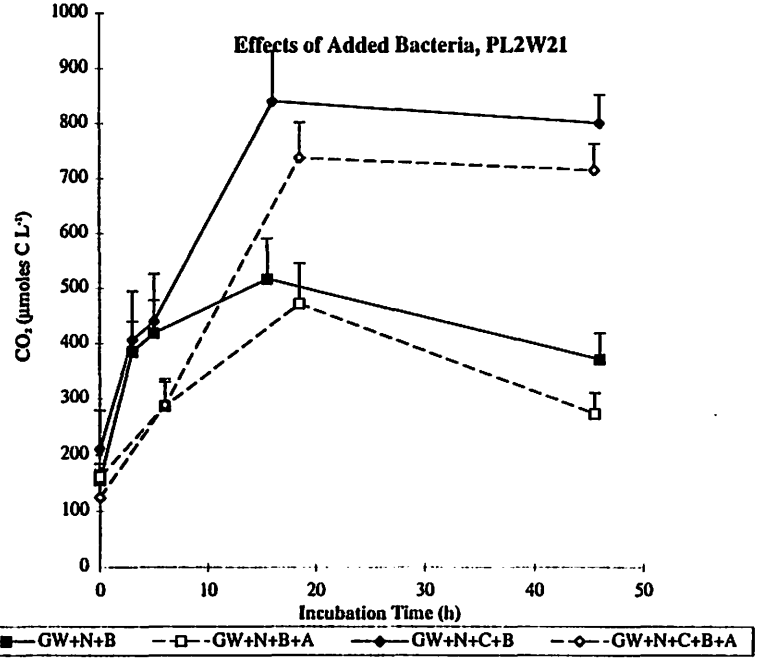
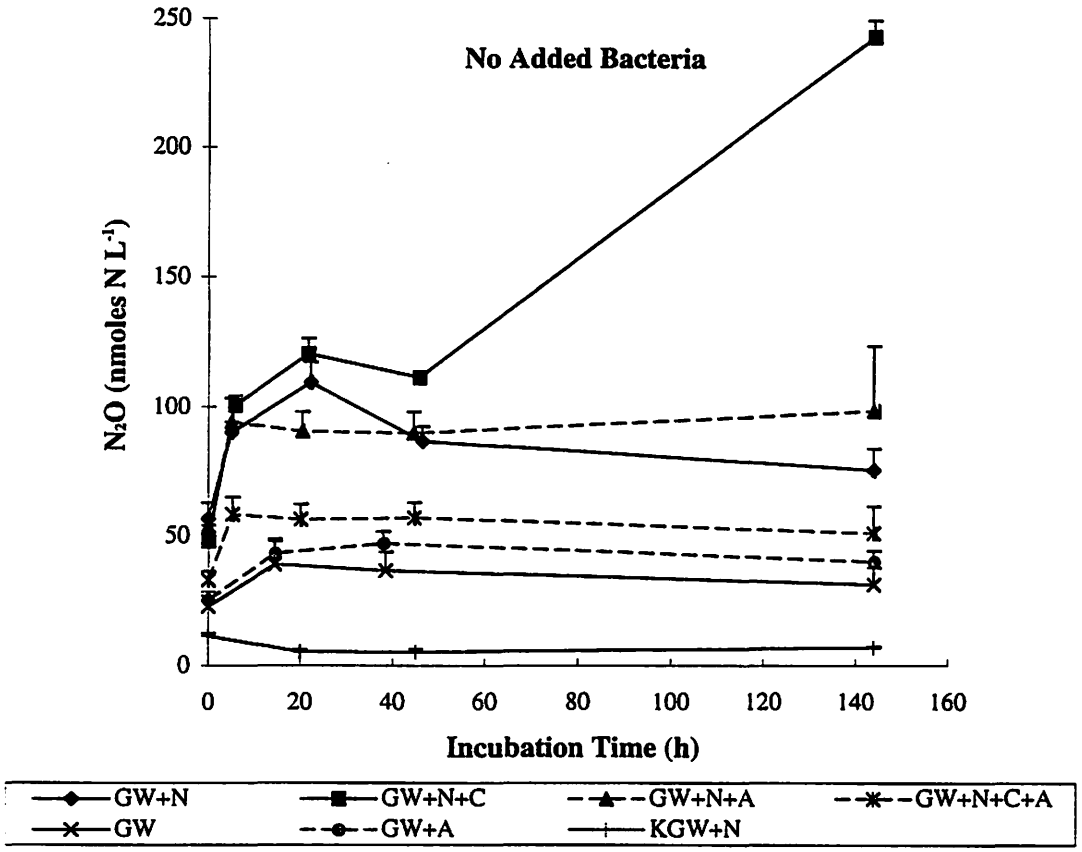


Figure 5. Denitrification, Well F3, April 1996



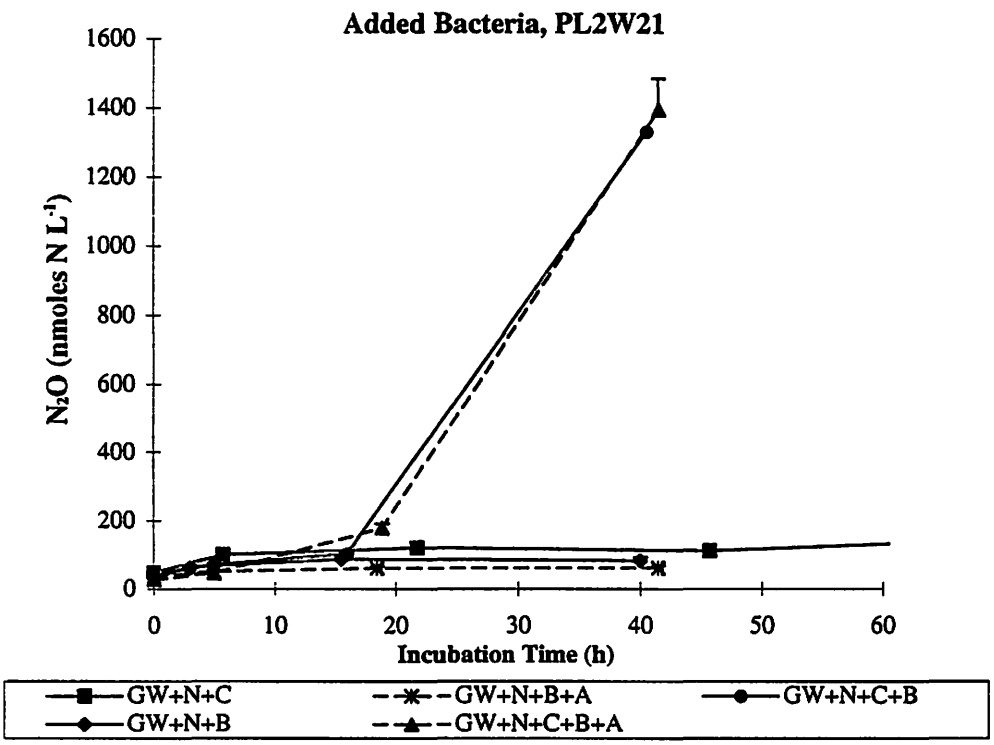


Figure 6. Dissolved Inorganic Nitrogen, Well F3, April 1996

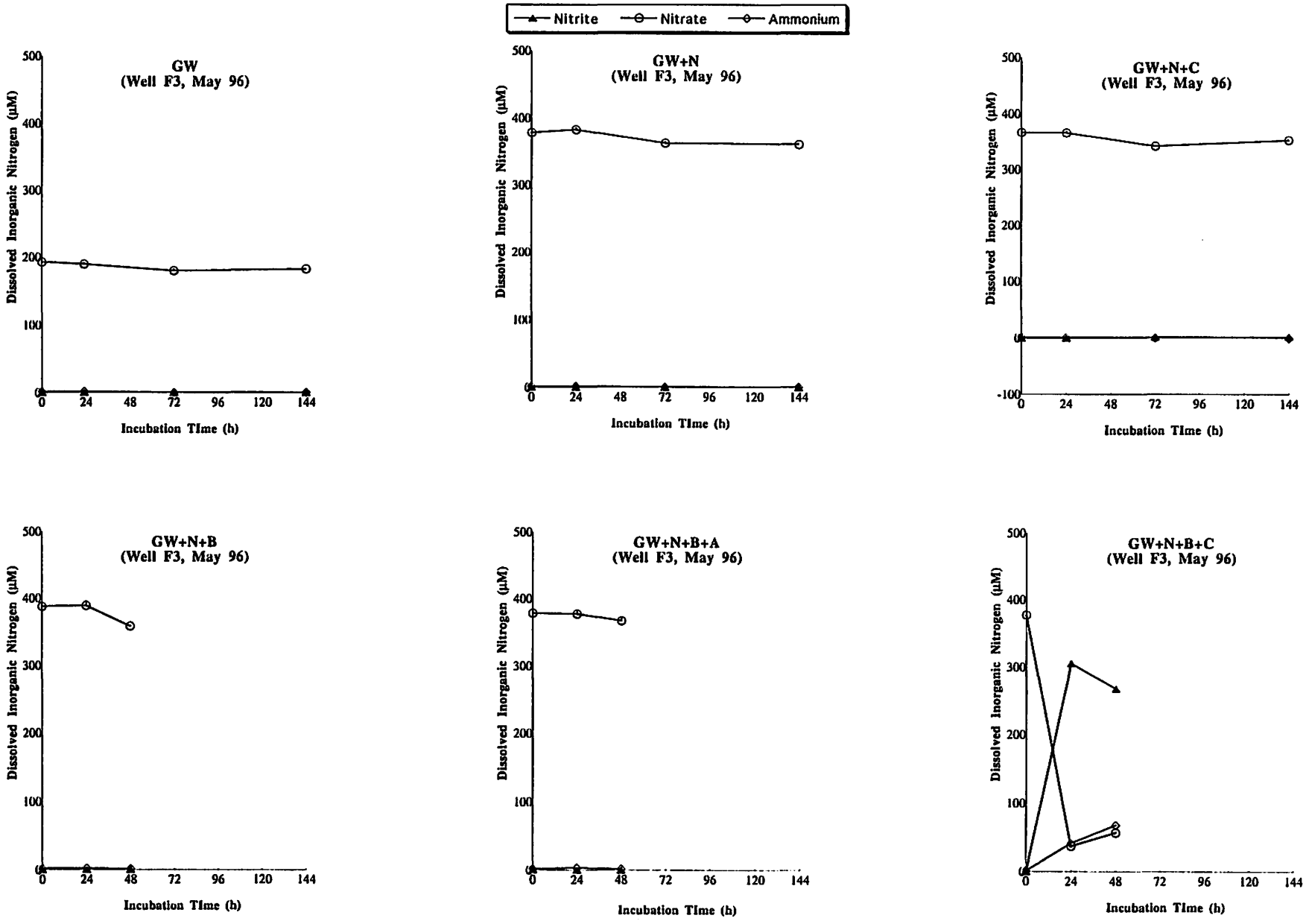


Figure 7. Dissolved Inorganic Nitrogen, Well F3, June 1996

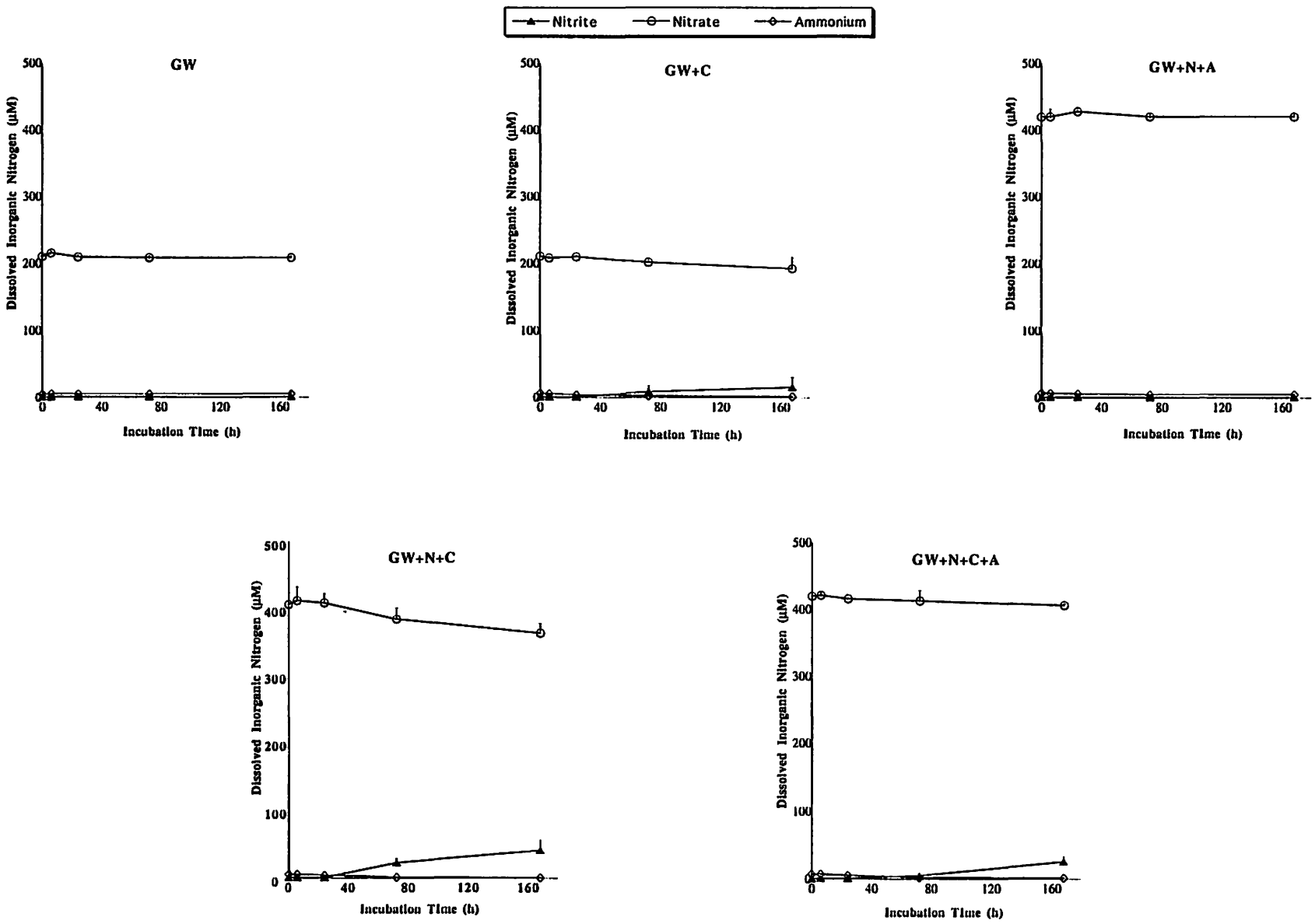


Figure 8. Dissolved Inorganic Nitrogen, Well D1, June 1996

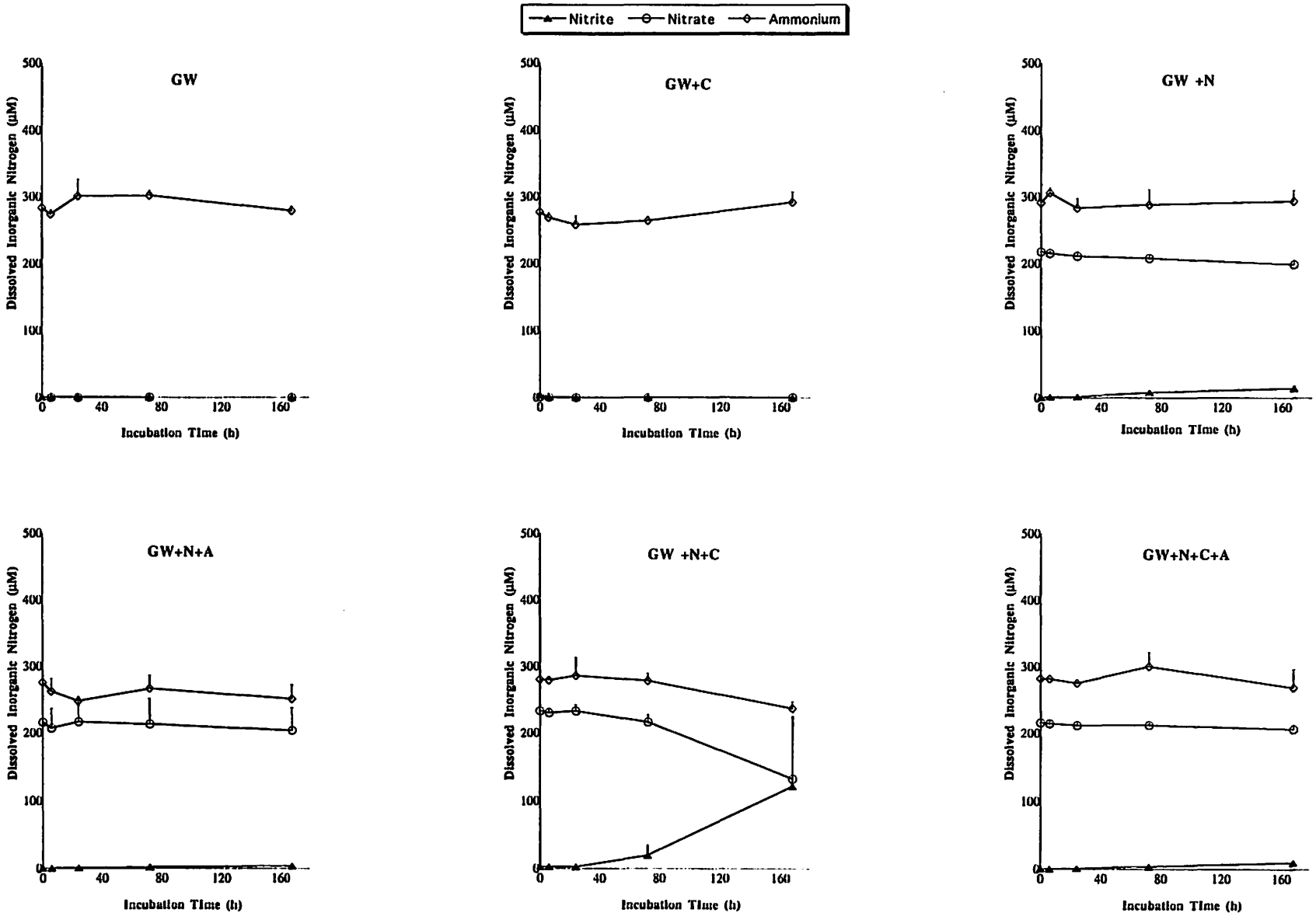


Figure 9. Respiration, June 1996

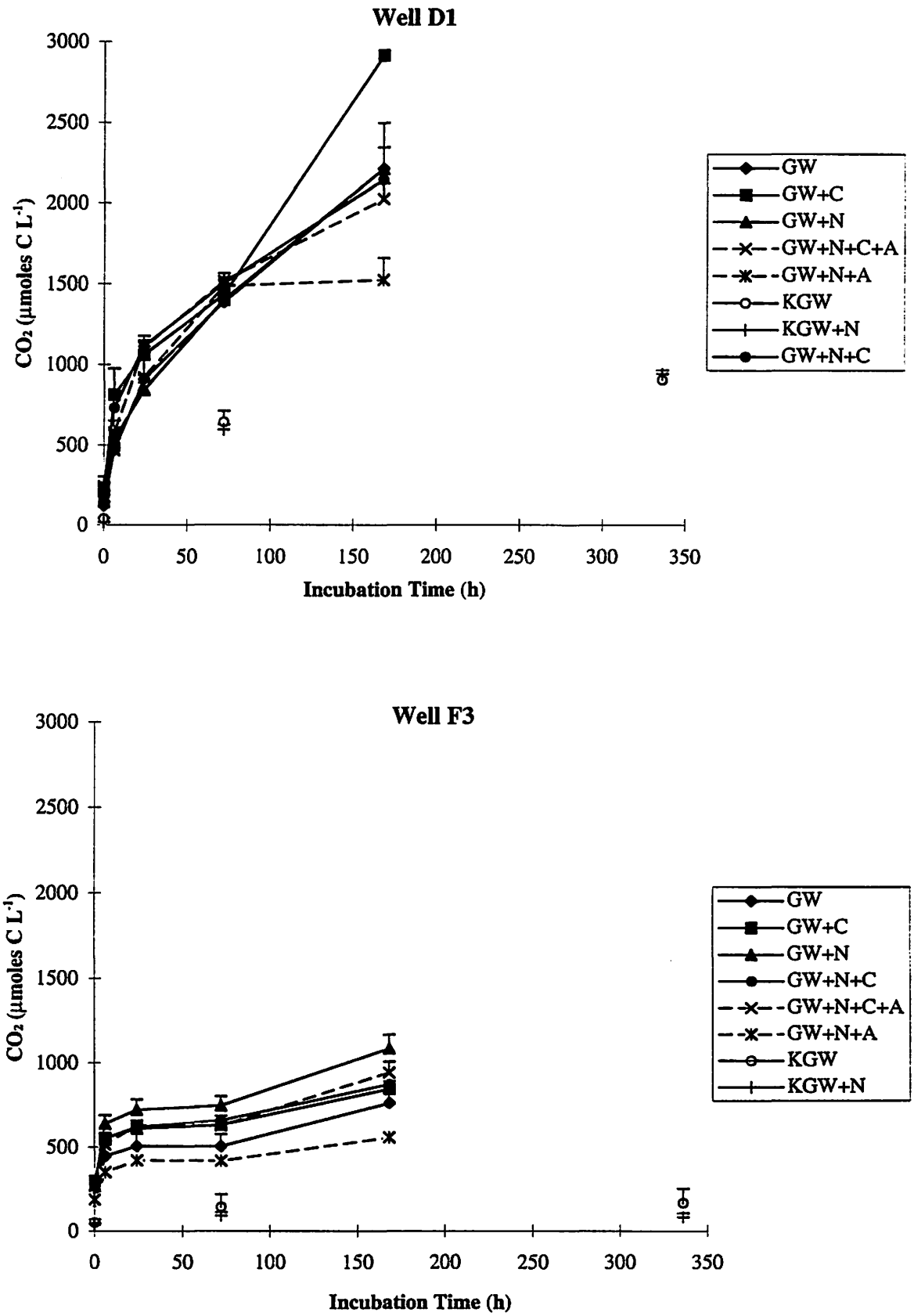


Figure 10. Denitrification, June 1996