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Development and application of a system for the analysis of atmospheric, water and sediment nitrogen and carbon

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Abstract

Eutrophication and climate change, key environmental concerns, are both linked to the carbon and nitrogen cycles hence the improved understanding of these cycles is essential. Currently, there is no system that simultaneously measures the fluxes of the three key gas phase products of nitrogen and carbon cycling (CO_2, CH_4 and N_2O) in submerged ecosystems with hourly time resolution. A “Lake-in-a-box” (mesocosm) was developed in the laboratory which allowed the monitoring of key components of the carbon and nitrogen cycles within the air, water and sediments. The approach is automated, simple and time efficient and novel in its ability to examine many different carbon and nitrogen compounds in all three physical component of the “lake”. Dramatic fluctuations in gaseous flux and the concentrations of overlying water and sedimentary carbon and nitrogen compounds were noted over a three week period. These were split into five distinct phases which were linked to changes in sedimentary N and C cycling. The results highlighted the important of links between the two cycles and supported recent studies showing that estuarine sediments can act as both a source and a sink of nitrogen.

Keywords

analysis, development, application, carbon, system, nitrogen, sediment, water, atmospheric, GeoQUEST, CMMB

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Development and application of a system for the analysis of atmospheric, water and sediment nitrogen and carbon

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Eutrophication and climate change, key environmental concerns, are both linked to the carbon and nitrogen cycles hence the improved understanding of these cycles is essential. Currently, there is no system that simultaneously measures the fluxes of the three key gas phase products of nitrogen and carbon cycling (CO_2, CH_4 and N_2O) in submerged ecosystems with hourly time resolution. A "Lake-in-a-box" (mesocosm) was developed in the laboratory which allowed the monitoring of key components of the carbon and nitrogen cycles within the air, water and sediments. The approach is automated, simple and time efficient and novel in its ability to examine many different carbon and nitrogen compounds in all three physical component of the "lake". Dramatic fluctuations in gaseous flux and the concentrations of overlying water and sedimentary carbon and nitrogen compounds were noted over a three week period. These were split into five distinct phases which were linked to changes in sedimentary N and C cycling. The results highlighted the important of links between the two cycles and supported recent studies showing that estuarine sediments can act as both a source and a sink of nitrogen.

Keywords: Nitrogen cycle, carbon cycle, monitoring systems, estuarine chemistry, gas production. Australia, New South Wales, Lake Macquarie.

Introduction

The carbon and nitrogen cycling ability of an ecosystem is often directly related to the health of the ecosystem and its ability to cope with environmental change. These cycles are intimately linked and play crucial roles in the production and consumption of key greenhouse gases, specifically CO_2, CH_4 and N_2O.

There are many different nitrogen species in the estuarine environment. The major components include organic nitrogen (OrgN, eg. amino acids, urea and proteins), nitrite (NO_2^-), nitrate (NO_3^-), ammonia (NH_3), ammonium (NH_4^+), nitrous oxide (N_2O), and nitrogen gas (N_2). The chemical transformations between these compounds are mediated through a series of chemical and biological reactions. These reactions are dependent on a variety of factors including oxygen availability. This series of processes, along with the exchange between the air, water and sediment phases, is commonly known of as the nitrogen cycle.

Two key processes within the nitrogen cycle, nitrification (the oxidation of NH_4^+ to NO_3^-) and denitrification (the reduction of $NO_3^-$to N_2), produce N_2O, a significant greenhouse gas, as a by-product. Carbon dioxide (CO_2) and methane (CH_4) are also important greenhouse gases. They play significant roles in the carbon cycle, which is coupled to the nitrogen cycle.

Traditionally, nitrogen cycling in coastal ecosystems has been found to be a sink of biologically available nitrogen. However, Fulweiler *et al.* [1] observed that estuaries could change from consuming inorganic nitrogen to fixing N_2, leading to estuarine areas acting as a nitrogen source rather than a sink. For the estuary studied by Fulweiler *et al.* [1], this switch was attributed to climate induced changes in primary production which lead to an increase in carbon based organic matter deposition.

The link between the carbon and nitrogen cycles, through the processes of primary production (the conversion of simple molecules to more complex ones) and organic matter remineralisation (decomposition of complex carbon and nitrogen compounds), has been known for many years (e.g. Kristensen and Blackburn [2]).

Studies have highlighted the influence of the C:N ratio within the sediment on nitrogen and carbon cycling [3]. Most researchers have focused on the role of nitrogen in primary production although some have examined it in light of methane oxidation [4]. Dodds *et al.* [5] found that the C:N ratio of the sediment can control the rate of nitrogen retention in the sediment.

Changes in various environmental parameters, including pH, salinity, oxygen and sediment organic carbon concentrations and sources, can also affect nitrogen cycling [6-8]. The elucidation of mechanisms of these effects has been impeded by the inability to examine the nitrogen cycle as a whole.

Previous studies have focused on one or two aspects of the cycle in isolation. For example, studies on denitrification [9] or diffusion into and out of the sediment [10]. Other researchers have examined the nitrogen cycle within a single phase of the environment, such as the sediment [11]. Membrane inlet mass spectrometry (MIMS) has been used to measure N_2 produced by

denitrification. Alternate techniques are required for other sections of the cycle [12]. Yet, processes within the nitrogen cycle are so closely interlinked that it is not always possible to partition them or investigate them in isolation. A far better approach is to quantify multiple nitrogenous compounds and nitrogen cycling processes concurrently. This requires the implementation of a range of techniques in a systematic manner.

In previous methods, multiple gas samples were collected by hand over a fixed interval of time and analysed individually on an external instrument. This entailed a large amount of manual handling and limited the studies time resolution. Older methods also often required different analysis techniques for each gas. For example, Liikanen *et al.* [13] determined fluxes weekly by collecting syringe samples which were then analysed using gas chromatography and three different types of detectors.

More holistic studies of nitrogen cycling have been made. For example, Blackburn & Henricksen [14] focused on sedimentary and aqueous nitrogen compounds (sediment OrgN, porewater NH_4^+, exchangeable NH_4^+ and NOx (NO_2^- + NO_3^-)), but did not include gaseous compounds. Eyre and Ferguson [15] focused on water nutrient concentrations measuring dissolved organic and inorganic nitrogenous species and N_2. However, sediment nitrogenous species and N_2O were not considered. Baird, Ulanowicz & Boynton [16] examined 36 nitrogenous components within the sediment and water, yet gaseous nitrogen species were not measured. Based on the literature, there has been no simultaneous quantification of the nitrogen cycle within the air, water and sediment of a coastal estuarine system.

Currently there is no system that simultaneously measures the three key gas phase products of nitrogen and carbon cycling (CO_2, CH_4 and N_2O) in submerged ecosystems and is able to observe their flux changes with hourly time resolution. This research aimed to fill that need. We constructed a prototype system, using current analytical techniques, which allowed the real-time quantification of multiple nitrogenous and carbonaceous species in the air, water and sediment. Details of the prototype and its application are presented here. In addition, the data obtained has

been used to examine the effects of sediment disturbance on the nitrogen cycle and subsequent interactions with the carbon cycle. This was done in an attempt to build a more complete understanding of the estuarine nitrogen and carbon cycles.

Materials and Methods

The Lake-in-a-box

A "Lake-in-a-box" (mesocosm) was constructed (Figure 1). The three physical phases of a lake system: air, water and sediment were recreated in the "Lake-in-a-box". The concentrations of a variety of carbon and nitrogen compounds (OrgN, NH_3, NO_2^-, NO_3^-, N_2O, OrgC, CO_2, CH_4, and CO) within it were monitored. The "Lake-in-a-box" consisted of a clean airtight polycarbonate box (34.5 x 23.5 x 13.5 cm) with three ports: a gas inlet, a gas outlet, and a water sample collection port. A layer of sediment was placed in the box which was topped with fresh seawater. The lid was placed on the box to enclose the air above the "lake". A small piece of tubing and a frit was attached to the gas inlet. A small plastic container was embedded in the sediment beneath the frit. This allowed the aeration of the overlying water without disturbing sediment.

The monitoring of the "Lake-in-a-box" was conducted using a system constructed around it, as described below. Three sample regimes were used: continuous (2 min intervals), daily (every 1–2 days) and initial/final samples (before and after the experimental run). Table 1 shows a summary of the sampling and analyses schedule along with the analytical technique used. Details of the analytical techniques and their calibration are described in the Analytical Techniques section.

System Design

The experimental system constructed around the "Lake-in-a-box" is shown in Figure 1. It consisted of the White cell mounted on the FT-IR, a flow restrictor (flow controller SCFH, King Instrument Company, USA), pump (Vacuubrand, Germany, MV2), two Resistance Temperature Detectors (RTD), flowmeter and two molecular sieves (HydroPurge II, Alltech, USA). These were joined using Teflon© and copper tubing.

Airflow around the system was controlled by a series of computer-controlled valves (Burkert© 0–8 Bar, Australia). Three air flow cycles were established- circulating, flushing and maintenance. During the circulating cycle, air travelled in a closed loop from the White cell to the "Lake-in-a-box" and back, passing through the flow restrictor, pump and flow meter. When flushing, laboratory air was drawn into the system through the molecular sieves entering the White cell and exited the system through an exhaust valve after passing through the flow restrictor, pump, "Lake-in-a-box" and flow meter. The system also had a maintenance cycle, where the White cell was isolated from the "Lake-in-a-box" and evacuated, and the spectrum of the empty cell was measured. The cell was then refilled with air drawn from outside through the molecular sieves.

Leak tests of this system were conducted using CH_4 as a tracer. CH_4 was added to the system and the CH_4 concentration monitored. These tests were conducted with the "Lake-in-a-box" empty as to avoid the effects of CH_4 dissolution into or production from the sediment or water. The concentration was found to be constant over the circulate cycle and returned to background concentrations during the flushing mode. No significant leaks were noted.

System implementation

The system was employed in two 23 day experimental runs, using sediment collected from Lake Macquarie. Lake Macquarie, New South Wales is Australia's largest coastal estuarine lake. The lake is located approximately 70 km north of Sydney and covers an area of ~110 km^2. The area surrounding the sample site was mainly residential, with industrial and agricultural enterprises located nearby. Surface sediment (water depth = 1 m) was collected and wet sieved on-site through 2 mm mesh to remove large debris. Sieved sediments were stored in clean plastic containers until use. Clean seawater (salinity of ~35 ‰) was collected from Towradgi, NSW, Australia.

The "Lake-in-a-box" was kept in a laboratory thermostated to 22 °C. Turbidity within Lake Macquarie in the areas of the lake near the sediment collection site is high [17-19]. Field studies of other estuarine systems at comparable water depths and turbidities observed PAR measurements

< 20 µmol/m^2/sec [20]. The PAR measured inside the "Lake-in-a-box" was similar ~15 µmol/m^2/sec (SpectraSense PAR Quantum Sensor, Skye Instruments Ltd).

At the start of each experiment, the sediment was vigorously mixed (to homogenise and simulate a sediment disturbance event), after which initial sub-samples were collected. The "Lake-in-a-box" was filled to a depth of 3–4 cm with sediment, and overlaid with 3–4 cm of clean seawater (from which initial sub-samples had already been collected). The lid was sealed at the start of the experimental run. The final water and sediment samples were collected at test completion. Gas phase analysis was implemented using an hourly cycle (20 min flushing, 40 min circulating). Maintenance spectra (empty cell) were collected once a day at midnight. Daily water samples were collected during the experimental run.

Analytical techniques

All glass- and plastic-ware were cleaned with detergent followed by three rinses with deionised water (Milli-Q, Millipore). All chemicals used in there analyses were analytical reagent grade or equivalent analytical purity.

Water Analysis

The analysis of all initial/final and daily water samples was conducted in triplicate. Blanks were carried throughout all experimental procedures. Multiple in-house gravimetrically prepared standards based on analytical grade $NaNO_2$ (AMPC fine chemicals), $NaNO_3$ (BHD AnalaR®) and Na_2CO_3 (99.9-100.1% pure, Ajax chemicals) were prepared for the analyses described below.

Nitrite concentrations were determined spectrophotometrically following method 4500–NO_2^- B [21]. Unless stated otherwise, all further methods were also obtained from Eaton *et al.* [21].

Nitrate concentrations were determined according to method 4110 A, using ion chromatography (Dionex ICS–90 Ion chromatograph, USA, with an IonPac® AS14A–5 µm 3*150 mm Analytical column and Peak Net software, buffer - 8 mM Na_2CO_3 + 1 mM $NaHCO_3$, suppressor – 0.1 N H_2SO_4).

Ammonia concentrations were determined titrimetrically using H_2SO_4 standardised against a gravimetrically prepared Na_2CO_3 standard following distillation (Methods 4500- NH_3 B and C).

Sediment and Porewater Analysis

Like the water analyses all sediment samples were analysed in at least triplicate and blanks were carried throughout all procedures. Sedimentary organic nitrogen was converted to ammonia using the Kjeldahl digestion and distillation method (4500-N_{org}). Sediment samples were distilled following method 4500- NH_3 B. Concentrations of ammonia in the distillates were determined following the procedure outlined previously for water samples.

Total organic carbon (TOC) analyses were conducted by an external laboratory (National Measurement Institute) using a high temperature TOC analyser (Dohrmann DC-190, USA). This followed the removal of inorganic carbon (carbonates and bicarbonates) by acidification with 1 M HCl (method 5310 B). The C:N ratio was calculated as the ratio between the average TOC and OrgN concentrations with an error calculated from the uncertainties in the TOC and OrgN concentrations.

Extractable nitrate and ammonia concentrations were determined using the extraction methods described by Hatton *et al.* [22] and Hatton and Pickering [23]. These were analysed following the techniques outlined previously for water samples. Extractable ammonia and nitrate concentrations are considered to represent the ammonia and nitrate loosely bound to the surface of sediment particles (i.e. readily released).

Porewater samples were collected by filling a 50 mL polycarbonate centrifuge tube with sediment. This was then capped and centrifuged (2500 rpm for 20 min). The porewater supernatant was filtered (<0.45 µm, 25 mm, SRP 25, Sartorius) and stored at <4°C. Porewater concentrations of OrgN, NO_2^-, NO_3^- and NH_4^+ were analysed using the same techniques described previously.

Gas Analysis

Gas phase concentrations of H_2O, N_2O, CO_2, CO and CH_4 were measured using Fourier Transform Infrared spectroscopy (FT-IR), as described by Esler *et al.* [24]. A Bomem MB100 FT-IR (Canada),

operating at 1 cm^{-1} resolution, fitted with a bandpass interference filter (pass band 2000–3300 cm^{-1}) and a liquid nitrogen-cooled Mercury Cadmium Telluride infrared detector (Judson technologies, USA) was used during the measurements. Air was analyzed in a multipass (White optics) cell with an optical path length of 21.5 m (Infrared Analysis Inc., USA, Model No. 31.5–V) which had been coupled to the FT-IR. A pressure transducer (MKS Instruments, USA, Baratron 122AA–01000AD) was used to monitor the pressure within the cell and a PT100 Resistance Temperature Detector (RTD) measured the temperature of the cell. The pressure and temperature of the cell were recorded using a computer program developed by Kettlewell (2003). This program also controlled the switching of the valves, timing and recording of the collected spectra.

The concentrations of H_2O, N_2O, CO_2, CO and CH_4 in the air were determined by a non-linear regression (fit) between the measured spectra and calculated spectra derived using the Multiple Atmospheric Layer Transmission program (MALT) [25] and the HITRAN dataset [26]. This non-linear fitting is an iterative method based on the Levenberg-Marquardt method [27]. The concentrations of each gas species and the instrumental lineshape parameters are varied until the "best fit" (minimum sum of the squared residual) is obtained. The use of standard infrared spectra databases instead of calibration gases is a well established technique and has been used in a variety of applications (e.g. Yokelson *et al.* [28]). The validity and calibration of this particular gas analysis technique has been examined previously [24, 29]. Griffith [29] details the analysis of eleven CSIRO GASLAB standards by this FT-IR technique. He found that the retrieved concentrations of CO_2, CH_4 and N_2O varied by less than 1.5 % from the GASLAB concentrations and were very precise (<0.25 %). The uncertainly in the optical path length will increase this error, typically by at most a few percent. These errors are expected to be <5 %. The gaseous flux was determined from the changes in these concentrations.

The method detection limits (MDL) and precision for each gas were determined. The MDL was calculated, following standard procedures, as three times the standard deviation of fluxes near

the expected limit of detection. The precision was calculated as an average percentage error of those fluxes not used to calculate the MDL.

The system was designed to allow sampling for N_2 as well using MIMS [12]. However, these measurements were not successful during this study due to technical problems with the mass spectrometer.

Error Assessment

The wet chemical techniques used in this study, ion chromatography, UV-Vis spectrophotometry, Kjeldahl nitrogen and titrimetric ammonia analysis are standard analytical techniques the validation of which has been examined in detail in other studies [21]. They found that the average error when analysing reference materials varied depending on the method. Errors for the ammonia method were the highest (up to 20 %) especially when high concentrations of organic nitrogen were in the sample. The average errors inherent in the other techniques used in this study were between 2 – 3 %. Organic carbon concentrations were determined by an external laboratory. They quoted recoveries between 80 – 100 %.

The error inherent in the method, as quoted in Eaton *et al.* [21], the standard deviation between each replicate sample and the standard error from the calibration curves (the scatter) were compared. The maximum of these values was given as the best estimate of the error.

Error in the gaseous flux was calculated based on the fit between the model and the data. It was larger than the maximum error (< 5 %) associated with the gas concentrations [29]. These errors are shown with the data in Figure 2.

Results

The two experimental runs obtained similar results. The first experiment was used to optimise the procedure leading to many interruptions in the data collection. Therefore, the second set is primarily shown here. The following section will present the data obtained during the experiments.

Initially the sediment was a consistent black colour. But, after 3 days distinct grey/brown (oxic) and deep black (anoxic) layers were clearly visible. White filamentous algae was observed on the surface of the sediment from day 7 of the experiment.

Figure 2 shows the changes in the overlying water concentrations of NO_3^- and NO_2^- and the gaseous flux derived from the changes in N_2O, CH_4 and CO_2 concentrations. The CH_4 flux peaked on day 3 and then decreased gradually throughout the remainder of the experimental period. A rapid increase in N_2O production began on day 9, and peaked three days later on day 12. More than 80 % of the N_2O produced was released from the sediments during days 10–15. The flux of CO_2 varied throughout the experiment, although a clear change occurred on day 7. CO_2 flux peaked on day 14, two days after the N_2O peak. Overlying water concentrations of NO_2^- peaked late on day 14 with the NO_3^- concentration of the overlying water peaking on day 21 (Figure 2).

Table 2 provides the TOC concentrations, C:N ratio and sediment moisture content. The concentration of organic carbon and moisture content were high. The C:N ratio was found to be >21. This ratio indicated that any ammonia produced within the sediment was not likely to be released into the overlying water, rather it would have remained within the sediment [3].

The experimental results for the nitrogen containing compounds were used to construct a nitrogen budget as shown in Table 2. This table allows the comparison of the concentrations of the various pools of nitrogen within the air, water and sediment and the total nitrogen mass for the system, before and at the completion of the study. The greatest (absolute) difference recorded over the 23 days was the OrgN concentration, which increased by approximately 20 %.

The mass of dinitrogen gas (N_2) evolved during the study could not be directly determined. Instead N_2 production was estimated from N_2O emissions (Table 2). Robinson, Nedwell, Harrison & Ogilvie [30] studied the emission of N_2O from a eutrophic estuary and found a maximum of 2 % of all denitrified nitrate was released from the sediment as N_2O. Similar results have been obtained in other studies of estuarine and marine sediments 0.1 – 0.4 % [31], 0.5 – 4 % [32] and 2 – 4 % [33]. Estuarine studies have found that the mass of N_2O produced during sedimentary nitrification

was insignificant in comparison to the mass produced during denitrification [30, 33]. Water column studies by de Wilde and de Bie [31] and Punshon and Moore [34] have found nitrification N_2O production rates of 0.07–0.42 % and 0.01 – 0.11 %, respectively. These are an order of magnitude smaller than denitrification production rates. Based on this literature, it seems sensible to assume that the vast majority of N_2O released from the sediment was due to denitrification and that the mass of N_2O is representative of approximately 2 % of all nitrate denitrified. The amount of N_2 released was estimated from this.

There was a significant ~20 % (P = 0.05), increase in sediment OrgN concentration from day 1 to day 23, and a significant decrease in porewater OrgN ~90 % (P = 0.05). The decrease in porewater OrgN was not large enough to account for the increase in sediment OrgN over the study period. The total mass of nitrogen within the system at the end of the study was significantly larger (P = 0.05) than the initial mass of nitrogen even before the estimated mass of N_2 produced is considered.

Table 3 shows the Method Detection Limits (MDL) and precision estimates for each gas.

Discussion

Data interpretation

To simplify the data interpretation, we have divided the "Lake-in-a-box" measurement period into 5 phases (Figure 2). Each phase corresponds to a notable alteration in nitrogenous or carbonaceous flux. We have then proposed a sequence of nitrogen and carbon cycling reactions for each of the phases (Figure 3).

Organic Nitrogen and Carbon

Organic nitrogen (OrgN) and carbon (OrgC) measurements were made at the commencement and conclusion of the experimental run. Sediment OrgN concentration increased by ~20 % over the study period, but there was no equivalent decrease in other nitrogen components. So this increase must be attributed to an unmonitored pool of nitrogen. This, we suggest, is the fixation of atmospheric N_2 by micro-organisms within the "Lake-in-a-box".

Nitrogen can be thought of as "biologically available" (NH_4^+, NO_3^-, NO_2^-, OrgN) or "biologically unavailable" (N_2). Estuarine sediments have been traditionally thought to act as a sink of biologically available nitrogen by absorbing NH_4^+, NO_3^-, NO_2^-, OrgN and releasing N_2 [35]. Fulweiler et. al. [1] in a recent paper challenged this assumption. They observed significant N_2 fixation in sub-tidal marine sediments. That is, the sediments acting as a net source of biologically available nitrogen. Our measurements support this.

Several factors can delay or inhibit N_2 fixation. As an anoxic reaction [36], N_2 fixation would have been delayed by the sediment disturbance, occurring on day 1, which would have introduced oxygen (O_2) deep into the sediments [37]. The N_2 fixing ability of the bacterial population would also have been impaired by the O_2 exposure of the sediment [38], as well as the darkness during sediment storage [39]. Disturbance is also known to cause a short term (min/hours) release of ammonia from the sediments to the overlying water [37] (Figure 3, Phase 1). This too can suppress nitrogenase activity and hence N_2 fixation [40]. For all these reasons it is expected that any N_2 fixation would begin after an initial delay. The increase in the OrgN content of the sediment corresponds with an increase in TOC concentration, indicating that primary production had occurred, a theory that is supported by the observed increase in algal biomass on the sediment surface. Biomass formation on sediment surfaces can influence carbon and nitrogen cycling, as is discussed in detail by Cook *et al.* [6, 41].

Strauss & Lamberti [3] proposed a critical C:N ratio of 21. Above this, any ammonia released from the sediment would be consumed within the sediment itself, rather than entering the overlying water. The C:N ratio of our sediments (34 ± 9) indicate that they were nitrogen limited. Hence, ammonia released from the sediments during the disturbance would not have been released to the overlying water but rather consumed within the sediment itself. No measurements of ammonia were made immediately following the disturbance of the sediment to confirm this, but future studies of this type will include these analyses.

Methane

Figure 2 shows the time dependence of methane (CH_4) fluxes; a single large peak is observed on day 3. CH_4 production within the sediment occurs in anoxic regions. Here facultative anaerobic bacteria use less energy efficient electron acceptors (OrgC) producing CH_4 [42]. The gradual increase in CH_4 production observed during the first three days can be linked to the sediment disturbance event which increased sediment O_2 penetration [37] diminishing the use of less efficient electron acceptors, and hence CH_4 production (Figure 2, Phase 1). However, as O_2 is progressively consumed anoxic regions reform, and CH_4 production gradually resumes. In addition, the diffusion of CH_4 from anoxic regions to the overlying air will delay the detection of CH_4 production (Figure 2, Phase 2).

A clear decline in CH_4 flux is observed commencing on day 3. It is unlikely that this decline is due to the exhaustion of the OrgC substrate in the sediment, as the TOC was high and did not decrease substantially during the study (Table 2) (although not all carbon is bio-available [8]). It is more likely that the CH_4 production remained constant while the activity of methanotrophic (CH_4 consuming) bacteria increased, leading to a decrease in the CH_4 flux (Figure 3, Phase 3). Methanotrophic bacteria produce CO_2 following CH_4 consumption. So, an accompanying rise in CO_2 flux would be expected [43]. However, due to the magnitude of the background CO_2 concentrations and large fluxes of CO_2 observed (~40 times larger than CH_4), such a small increase in CO_2 release from the sediment would not be detected.

The disturbance induced sedimentary release of ammonium would have impeded methanotrophic CH_4 consumption in the early stages of the experiment. Methanotrophic bacteria will preferentially nitrify (oxidise) NH_4^+ to NO_2^- [44] producing N_2O as a by-product [43]. Consequently, a release of NH_4^+ from the sediment surface into the porewater could cause a decrease in CH_4 consumption. This would result in an increase in the CH_4 flux (Phase 2, Figure 2). Once methanotrophic bacteria have consumed any available NH_4^+ they would consume the next available electron acceptor, in this case CH_4, leading to a decrease in CH_4 flux (Figure 3 Phase 3).

Nitrous Oxide

As indicated by the overall increase in the total sedimentary nitrogen content (Table 2), a significant amount of nitrogen has entered the sediment. Yet, the large volume of N_2O produced during Phase 4 and Phase 5 shows that nitrogen has also been lost. Nitrous oxide is a by-product of both nitrification and denitrification. However, the lack of NO_2^- build up in the overlying water during the N_2O production period (Days 8–23) suggests that nitrification was not the dominant source.

The large volume of N_2O released from the sediment originated from four possible pools. These are: the ammonia released from the sediment due to disturbance, the nitrification of which would produce a second nitrogen pool; OrgN originally present within the sediment; and N_2 fixed during the experiment. The following section discusses the likelihood of these pools being the dominant source of N_2O.

The ammonia released from the sediment by the initial disturbance event could promote N_2O formation from either methanotrophic or non-methanotrophic nitrification. We suggest three reasons that the N_2O produced from these two pathways would be too small to be observed. Firstly, the mass of N_2O produced via nitrification in estuarine sediments is known to be insignificant in comparison to denitrification [30, 33]. Secondly, the production of N_2O from methanotrophic nitrification is known to be even lower than that of traditional nitrifies. For example, Yoshinari [45] found that the maximum production of N_2O by methanotrophic bacteria is only 1.6 % of the maximum production rate for Nitrosomonas europaea, a common nitrifying bacteria. Also, the timing of the N_2O peak (days 9–15) does not correspond with that of the sedimentary ammonia release (days 0–1). Hence the nitrification of the released ammonia is not expected to be a dominant N_2O source.

Another source to consider is the pool of nitrite produced from the nitrification of the ammonia released from the sediments. This could be subsequently oxidised and denitrified. Increases in overlying water nitrite and nitrate concentrations were not observed until day 9, suggesting that this pool remained within the sediment (Figure 3 Phase 2). Any denitrification (reduction of NO_3^- to N_2) of this pool would produce N_2O [46], making this a possible N_2O source.

Denitrification is expected to be a stronger source of N_2O (see previous discussion). However, we suspect that the denitrification of this particular nitrogen pool is not a significant N_2O source. The maximum size of the pool was estimated by assuming that all the NH_4^+ was converted to NO_3^- and was available for denitrification. This is not necessarily true, as other reaction pathways will compete for the pool of inorganic nitrogen. The short term nature (min-hours) [37] of the sedimentary ammonia release would also limit the amount of ammonia available for denitrification. The reported percentage of all nitrate processed via denitrification and released from estuarine sediment as N_2O ranges from 0.1 – 4 % [30-33]. Using this range, the maximum mass of N_2O released from the sediment by the processing of this pool was estimated. It could only account for 50 % of the total N_2O released from the sediment. Hence, this pool could not be the sole source of the N_2O produced during the experimental run. Considering the limitations mentioned previously and the timing of the event it is not even likely to be a major source.

Thirdly, the processing of OrgN initially present within the sediment would produce N_2O, but as shown in the nitrogen budget (Table 2), the total sediment nitrogen content increased during the study. N_2O emissions could come from this expanding biomass. However, by examining the nitrogen budget alone we can not a priori separate this into pools of pre-existing and newly formed OrgN.

The timing of this N_2O flux indicates that the majority of the N_2O was sourced from fixed N_2. Sedimentary N_2O production commences on Day 8, presumably when a significant source of nitrogen became available. As this nitrogen source appears unavailable for the first week of the experiment it is unlikely to be the OrgN originally present in the sediment or even the ammonia released from the sediment during the initial disturbance. It is far more likely that this nitrogen came from a source which was not available at the commencement of the experiment. We suggest that this is N_2 fixed during the experiment and that the initial delay was due to the disturbance induced increase in sedimentary O_2 concentration (discussed previously). At first glance, the processing of this freshly fixed N_2 does appear to be a rather rapid turn over but it is feasible.

Cyanobacteria are known to be the predominant N_2 fixer at the sediment water interface in the marine ecosystem [47] and are likely to play a significant role in the "Lake-in-a-box". These bacteria have been observed to rapidly release >50 % of all fixed N_2 as either NH_4^+ or dissolved OrgN [48, 49]. This rate climbs to 80–90 % during periods of rapid growth [50]. So it is reasonable to expect that NH_4^+ or dissolved OrgN would become available once N_2 fixation commenced, especially during the initial growth phase. This would be rapidly processed leading to the sedimentary release of N_2O. Thus, the changes in the nitrogen budget along with the timing of the N_2O flux indicate that the processing of recently fixed N_2 is the dominant source of N_2O.

This stimulation of nitrogen processing would lead to growth in the population of nitrogen processing (denitrifying, nitrifying and nitrogen fixing) bacteria. The initial mass of available labile OrgC would limit this growth, as OrgC produced by photosynthesis (the only carbon source) would be trapped in the algal biomass. Once this OrgC had been depleted, the nitrogen processing bacterial populations would no longer be able to grow and would begin to 'starve', die and decompose (Figure 3 - Phase 5). It is expected that as time progressed a steady state of nitrogen processing would be reached, where N_2 fixation was equal to the nitrification and denitrification driven N_2 and N_2O fluxes.

Nitrate and nitrite

The concentration of NO_2^- in the overlying water peaks late in the experimental run (day 14) two days after the N_2O peak. This timing indicates that the NO_2^- in the overlying water came from the nitrification of newly fixed N_2, not from the OrgN or inorganic nitrogen originally in the sediment. The increase in NO_3^- concentration within the oxygenated overlying water coincides with a decrease in the NO_2^-. This is broadly consistent with the conversion of the NO_2^- to NO_3^- released from the sediments within the oxic environment of the overlying water.

Technique

This research has successfully developed and trialled a prototype system that identified and quantified significant variations in the N_2O, CO_2 and CH_4 flux from estuarine sediments. Large

variations in the concentration of nitrogenous compounds within the overlying water, sediment and interstitial waters were also observed (Figure 3 and Table 2). These results clearly demonstrate the abilities of this system to rapidly quantify multiple nitrogenous and carbonaceous species in the air, water and sediment continuously over extended periods. This data also enabled the identification of clear changes in gaseous flux and the pools of aqueous and sedimentary nitrogen and carbon. Hence, the construction of the system and its application was successful.

Previous methods were labour intensive and time consuming. The method described in this study is not. Infrequent sampling can limit the time resolution of a study causing short term or pulse events to be missed. But this method allows the simultaneous determination of hourly flux rates of infrared active gases. The exact precision achievable will be limited by the surface area and the volume of the headspace used. The automated and continuous nature of this new method also allows studies to be conducted for prolonged periods, weeks or months.

The Method Detection Limits (MDL) were found to be well below the majority of the fluxes measured in this study (Table 3). The precision of the method (3–6 %) is a significant improvement over those previously reported [51, 52]. The MDL and precision estimates reported here are based on an hourly cycle. Increasing the cycle length would improve the MDL; however this would lower the measurement frequency. As such, there is a trade off between the MDL and the measurement frequency. Increases in the sensitivity of the detector and improvements to the accuracy of the FT-IR instrument could also lower the MDL and improve the precision of the method.

Conclusions

This research developed and applied a prototype system to study the nitrogen and carbon cycles within the aquatic environment. The prototype system allowed for the monitoring of gaseous, aqueous and sedimentary phase carbon and nitrogen species that are significant and pertinent to the study of nitrogen and cycling.

These results also served to demonstrate the ability of the prototype to monitor carbon and nitrogen cycling. The rapid, continuous and automated nature of this technique is a significant improvement over traditional flux measurement techniques. The system also significantly decreases sample handling, which means that measurements can be made far more frequently (on the hourly scale) and over long periods. The success of these *ex-situ* trials will lead to future applications of the system both in corer reactor systems and *in-situ* real-time in the field.

Recommended future improvements to the systems include the addition of daily aqueous ammonia measurements, N_2 gaseous measurements and light control, which would allow for an even greater understanding of the processes within the carbon and nitrogen cycles. The ability to monitor other parameters including pH, dissolved oxygen and water and sediment temperature would also be advantageous.

Significant changes in the gaseous fluxes of CH_4, N_2O and CO_2 and the overlying water concentrations of NO_2^- and NO_3^- were observed during the experiments. These included the production of N_2O and CH_4 in addition to increases in the OrgN content of the sediment. The exact mechanisms behind these changes are unclear although a series of explanations have been proposed. These highlighted the relationship between sedimentary nitrogen processing, oxygen availability and carbon cycling. The ability of sediments to act as both a source and sink of biologically available nitrogen was also evident.

Acknowledgments

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Table 1. Analytes and methods used in air, water and sediment analysis. Daily samples were collected every 1 – 2 days, continuous samples were collected at 1 min intervals and initial/final samples were collected before and after the experimental run. The analysis of all initial/final and daily sediment and water samples was conducted in triplicate.

Sample matrix	Sampling regime	Analyte	Method*
Air	Continuous	N_2O, CO_2, CO, CH_4	Fourier Transform Infrared Spectroscopy
Water	Daily	NO_3^-	Ion chromatography
	Daily	NO_2^-	Spectrophotometric method
	Initial/Final	NH_3	Distillation
	Initial/Final	Organic Nitrogen	Digestion and distillation
Porewater	Initial/Final	NO_3^-	Ion chromatography
	Initial/Final	NO_2^-	Spectrophotometric method
	Initial/Final	NH_3	Distillation
	Initial/Final	Organic Nitrogen	Digestion and distillation
Wet Sediment	Initial/Final	NH_3	Distillation
	Initial/Final	moisture content	Heating >24 hr at >100 °C
	Final	Total Organic Carbon	Infrared analysis
	Initial/Final	Organic Nitrogen	Digestion and distillation
Dry Sediment	Initial/Final	Extractable NO_3^-	Extraction/Ion chromatography
	Initial/Final	Extractable NO_2-	Extraction/Spectrophotometric method

* Please refer to the text for further information as to method specifics.

Table 2. Nitrogen budget and other parameters for the "Lake-in-a-box". All results are ± 1 SD. Detection limits are either twice the standard deviation of replicate blank samples or as given by Eaton *et al.* [21]

Nitrogen Budget		
	Initial (ppm–N)	*Final (ppm–N)*
Sediment		
OrgN	470 ± 10	550 ± 30
NH_3	12 ± 2	6 ± 1
Porewater		
OrgN	19 ± 5	<2
NH_3	3.2 ± 0.6	5 ± 1
NO_3^-	<0.1	<0.1
NO_2^-	<0.01	<0.01
Water		
OrgN	<0.1	<0.1
NH_3	<5	<5
NO_3^-	<0.1	<0.1
NO_2^-	<0.01	<0.01
Gaseous		
Mass of N released as N_2O	-	1.83 ± 0.03
Mass of N released as N_2[a]	-	92 ± 2
TOTAL	510 ± 10	650 ± 30

Sediment	Initial	Final
Moisture Content	59.5 ± 0.9 %	56.45 ± 0.01 %
TOC (mg/kg (Dry))	40000 ± 10000[b]	50000±10000
C:N ratio	34 ± 9	40 ± 8

[a] Assuming N_2O release is approximately 2 % of N_2 released
[b] Estimate range based on samples collected from the same site

Table 3. Method Detection Limits and Precision estimates for the calculated flux of each Gas. The detection limit was calculated as three times the standard deviation. The precision is the average calculated uncertainty retrieved for each hourly flux. See text for further details.

Gas	**Detection Limit (nmol/m^2/min)**	**Precision**
CO_2	1000	6 %
N_2O	0.3	3 %
CH_4	3	3 %

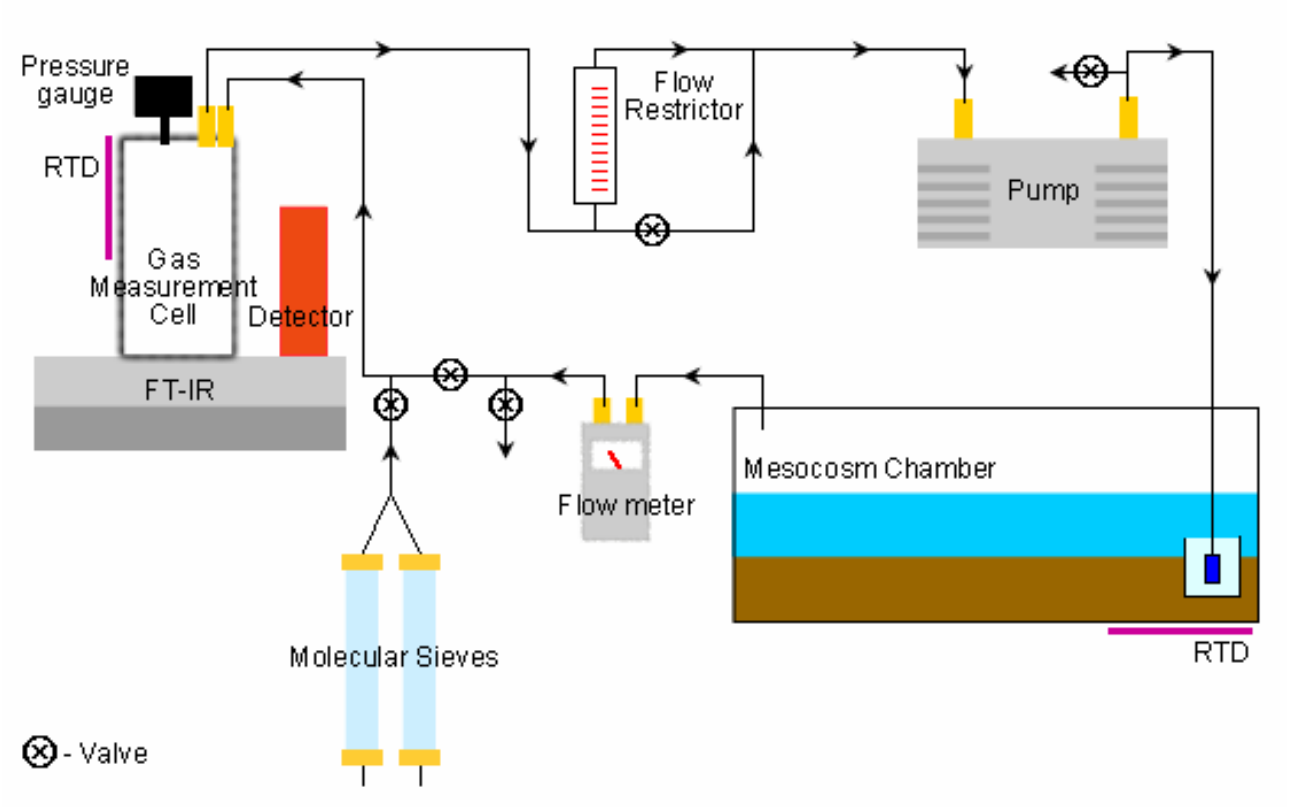
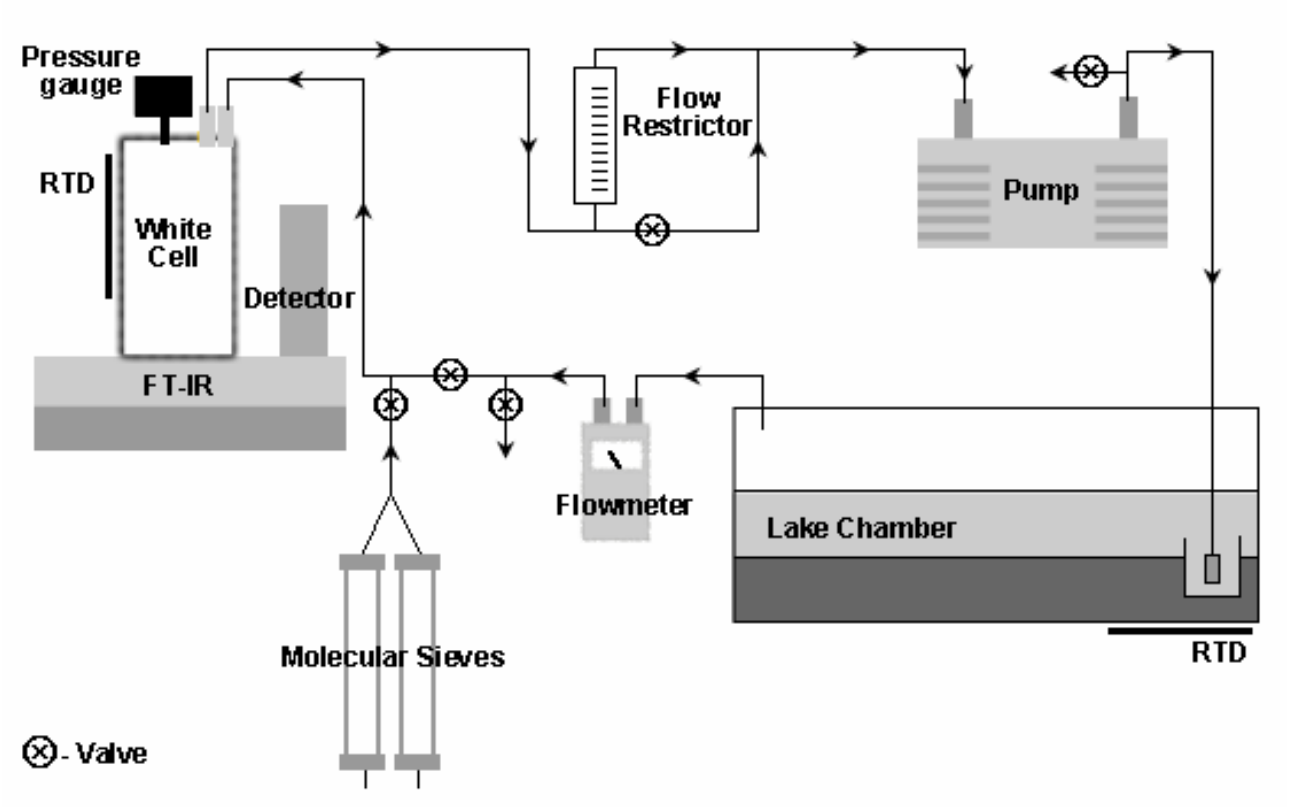


Figure 1. A circulating and flushing system consisting of the White cell of a Fourier transform infrared spectrometer (FT-IR), a flow controller, pump, lake chamber, two Resistance Temperature Detectors (RTD), flowmeter and molecular sieves joined using Teflon and copper tubing. The airflow is controlled by a series of computer-controlled valves. During the circulating cycle air travels in a closed loop from the White cell to the lake chamber and back passing through the flow controller, pump and flow meter. When flushing, ambient air is drawn into the system through the molecular sieves entering the white cell and leaving the system through a valve after passing through the flow controller, pump, lake chamber and flow meter. During background cycles the White cell was isolated from the lake chamber and evacuated by closing the valve above the molecular sieves and the valve between the flowmeter and the White Cell and opening the valve above the pump. The cell is then refilled with air drawn through the molecular sieves.



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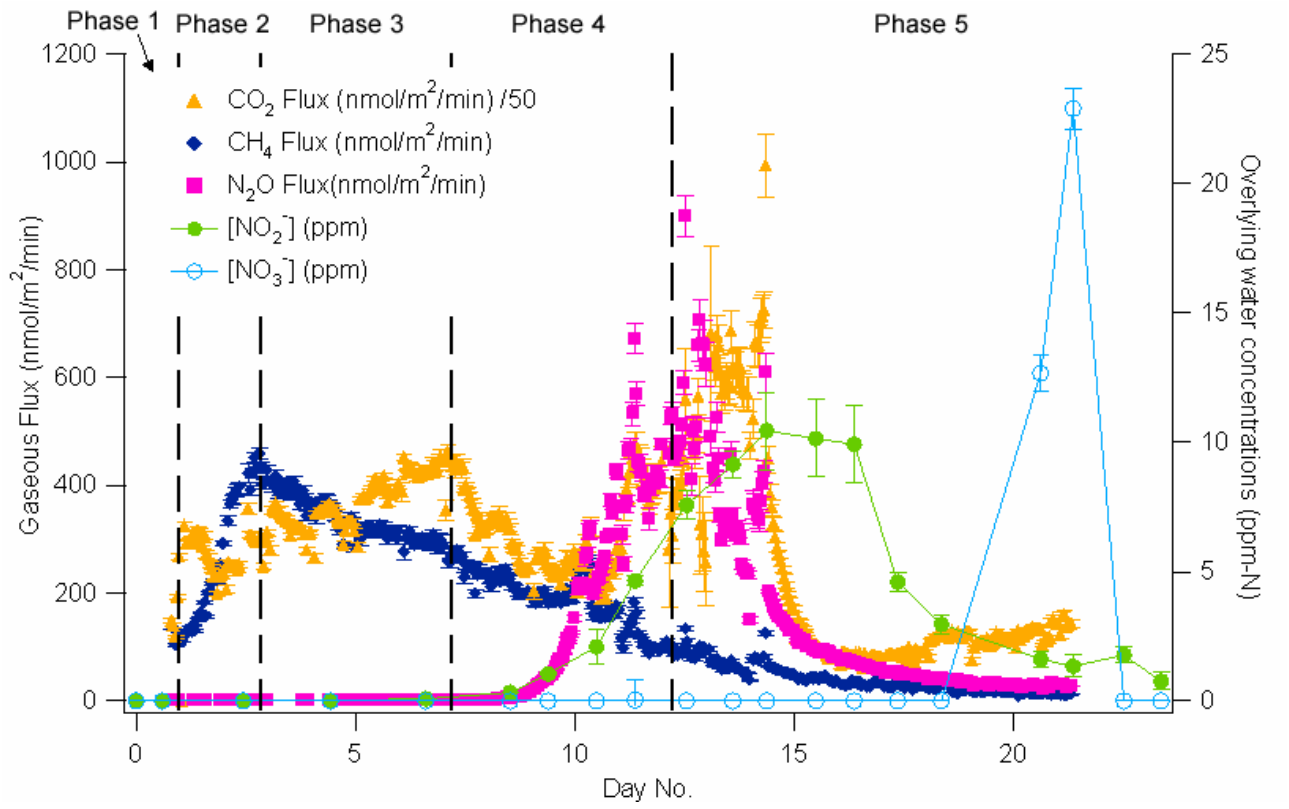
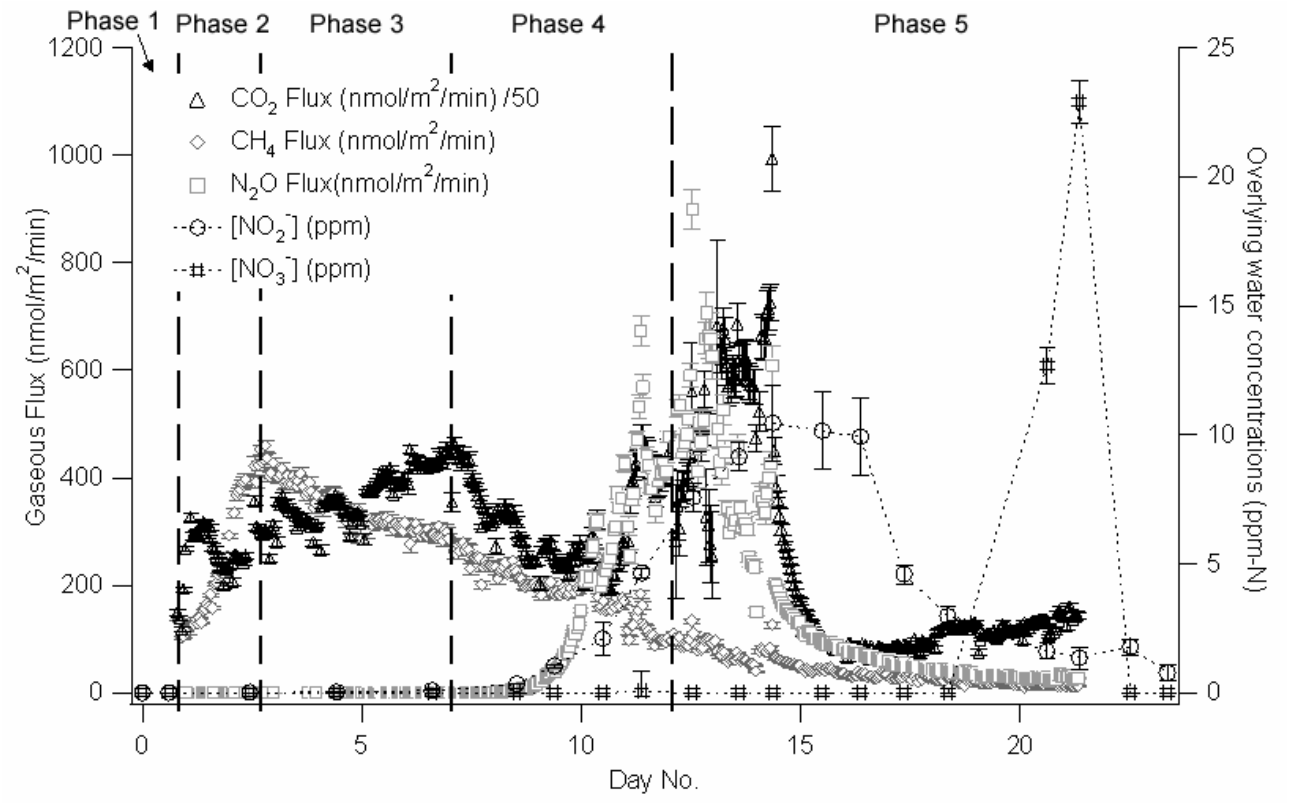


Figure 2. N_2O, CO_2 and CH_4 fluxes as measured in the headspace and NO_3^- and NO_2^- concentrations in the overlying water of the mesocosm during experiment. The phases shown are used in Figure 3.



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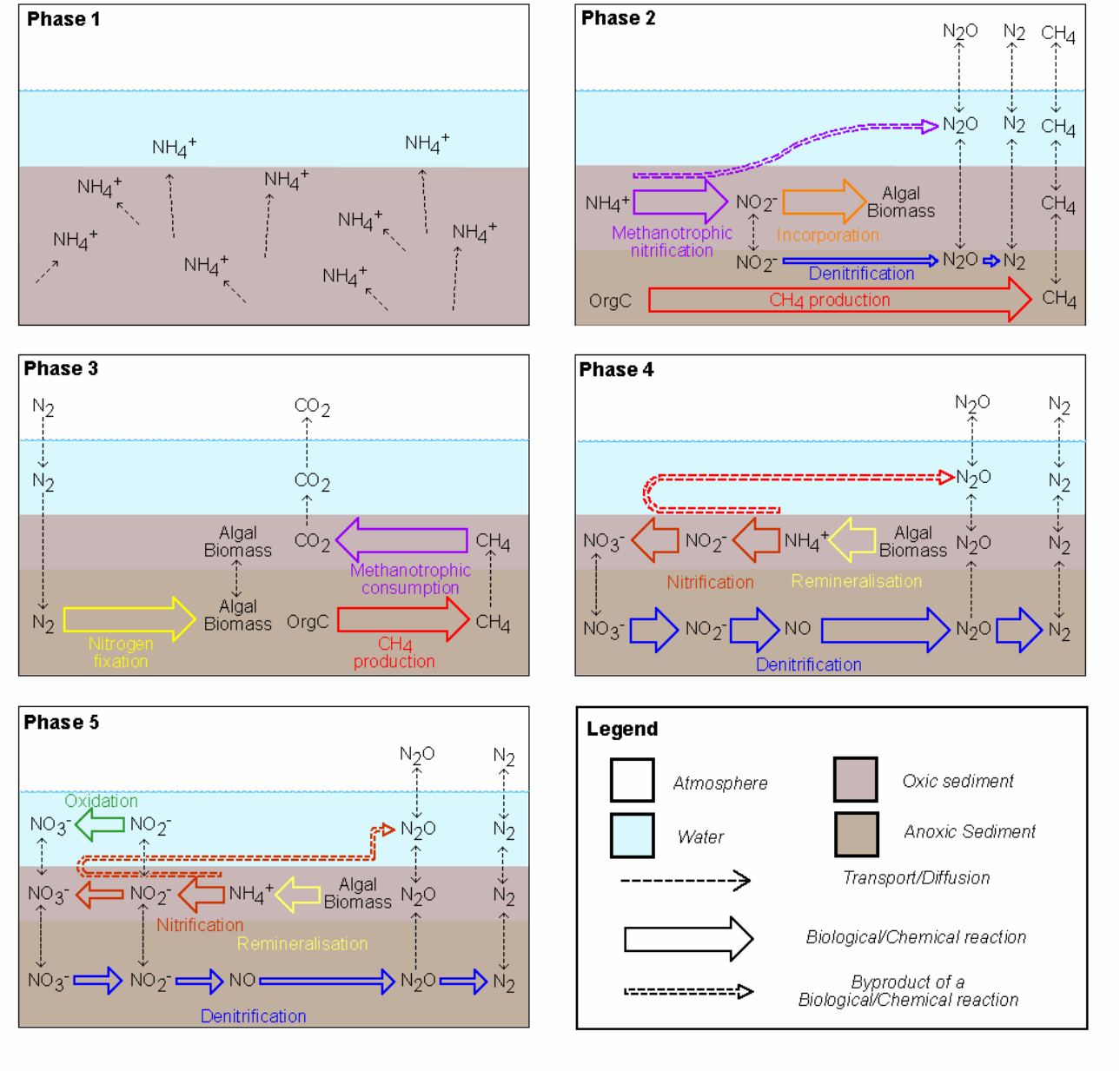
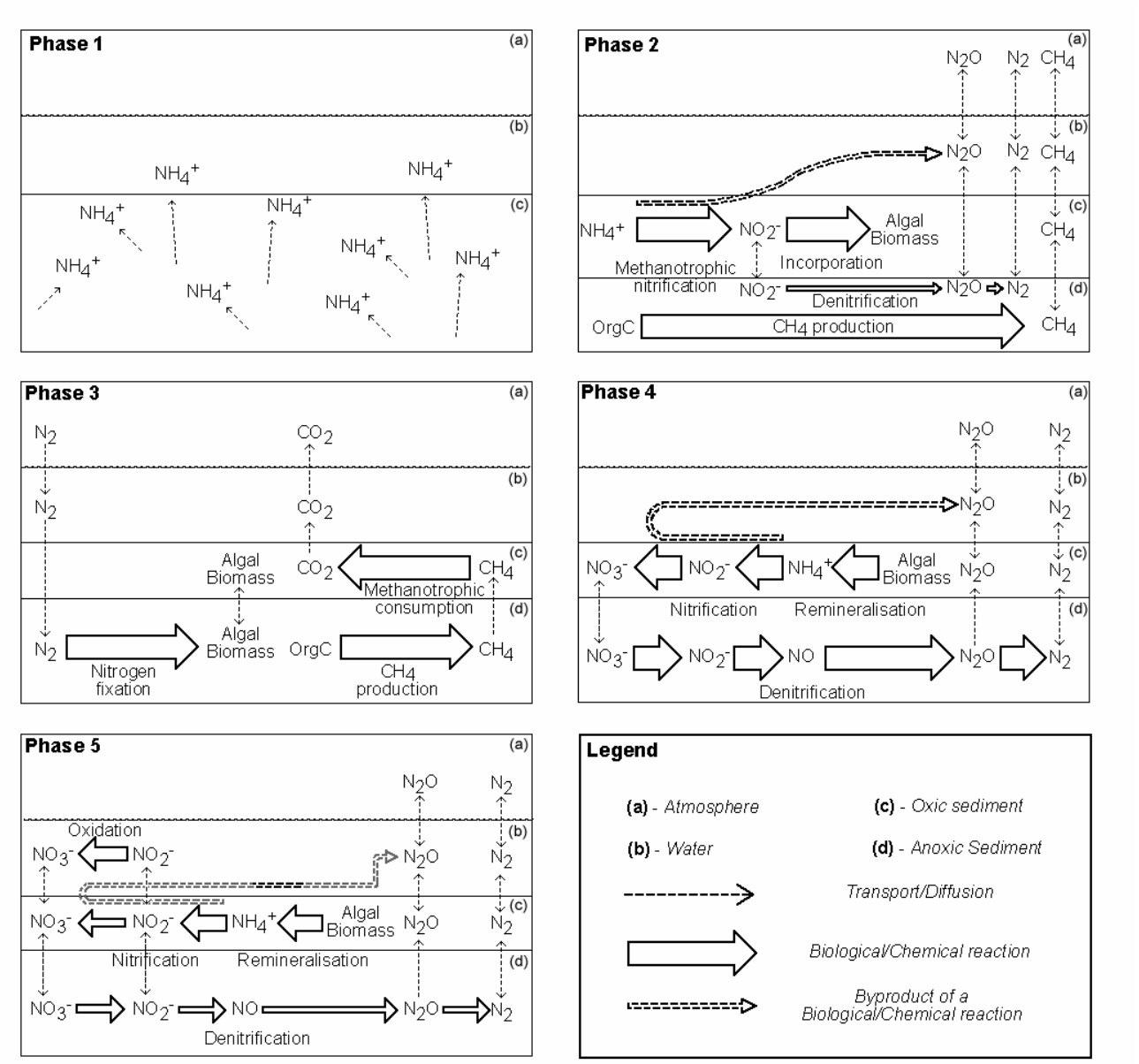


Figure 3.The chemical and biological reactions occurring during the experimental period. Phase 1- Disturbance oxygenates sediment and stimulates NH_4^+ release and inhibits CH_4 and N_2O production, Phase 2 - Anoxic areas begin to reform, CH_4 production and methanotrophic NH_4^+ consumption commence, Phase 3 - Methanotrophic CH_4 consumption and atmospheric nitrogen fixation commence, Phase 4 - The nitrification and denitrification of N_2 fixed from the atmosphere and released from the algal biomass commences producing N_2O and N_2, Phase 5 - Available labile carbon limits nitrogen cycling, reducing N_2O and N_2 production and releasing NO_2^- which is oxidised in the water layer to NO_3^-. For the periods of the Phases see Figure 2.



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