

Theory and operation of continuous flow systems for the study of benthic-pelagic coupling

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ABSTRACT: Continuous flow systems have not been widely used in the study of benthic-pelagic coupling in marine systems. This paper discusses the theoretical and practical use of continuous flow systems for the study of benthic exchange processes and presents the results of experiments which compared continuous flow (open) and closed (batch) systems and investigated the sensitivity of exchange rates to residence times in a continuous flow system. Continuous flow systems minimize the problem of environmental dependency of benthic-pelagic exchange rates by maintaining initial experimental conditions throughout an incubation. However, the selection of a supply rate (i.e. residence time) is critical in their operation. Variable residence times delineate 3 patterns of sediment-water exchange: a linear response which reflects an optimal supply rate, a feedback response which indicates deviation from initial experimental conditions (environmental dependency), and a washout response which results in an erroneous estimate of exchange. For either closed (batch) or continuous flow incubations, only linear responses result in valid estimates of sediment-water column exchange. Sediment oxygen consumption and benthic fluxes of NH_4 , NO_2 , PO_4 (1 station), and Si(OH)_4 (both stations) were significantly greater when measured using continuous flow methodology than when using closed (batch) incubations. Sediment oxygen consumption as well as fluxes of $\text{NO}_3 + \text{NO}_2$ varied directly with supply rate, while fluxes of NH_4 were not significantly different over the range of supply rates tested. Examinations of overlying water nutrient concentrations indicated that observed differences in benthic fluxes between the 2 methods and the varying sensitivities of nutrient flux to supply rate were a function of the residence time of the overlying water in the core. Rates were similar when both techniques resulted in linear responses. Differences between techniques resulted when either feedback or washout responses were observed. The observed variable response among nutrients indicated that the kinetics of benthic regeneration differ among nutrients and implies that operation of continuous flow systems (i.e. residence time) should be optimized for the analyte under study. Open (continuous flow) systems have many advantages for the determination of benthic-pelagic exchange rates. In a continuous flow system, initial experimental conditions are maintained throughout an incubation. This permits experiments of relatively long duration which allows increased statistical rigor and permits the direct study of cause-effect relationships through the use of experimental manipulations. The primary disadvantage of continuous flow systems lies in their labor-intensive operation.

KEY WORDS: Benthic-pelagic coupling · Methodology · Experimental manipulation · Chemostat · Nutrient flux · Sediment oxygen consumption · Continuous flow · Open system

INTRODUCTION

Benthic exchange processes such as sediment oxygen consumption and nutrient fluxes have been measured using a variety of methods. Indirect estimates of

benthic exchange rates have been determined using concentration gradients (Emerson et al. 1984, Westerland et al. 1986, Devol 1987, Helder & Andersen 1987, Klump & Martens 1989). These estimates are generally considered to be reliable for environments in which diffusion is the dominant process, as in deep sea sediments or those sediments characterized by low flow energies, minimal faunal activity and low depositional rates (Guinasso & Schink 1975, Berner 1976). Direct

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estimates of benthic exchange rates have been made using an upstream-downstream or flume technique (Dame et al. 1984, Sornin et al. 1990), but have most commonly been made using *in situ* or microcosm techniques. *In situ* experiments enclose water over sediment using domes, bell jars, plastic bags or other chambers while microcosm experiments involve the removal of intact sediment cores and overlying water for shipboard or laboratory incubation. Conventional microcosm approaches have used primarily closed, or batch, system techniques (Pomeroy 1959, Teal 1962, Zeitzschel 1980, Fisher et al. 1982, Boynton & Kemp 1985, Teague et al. 1988). In closed system techniques, sediment and overlying water are isolated from exchange with the surrounding environment and fluxes are determined by following the concentration change over time. In contrast, open system techniques permit exchange with the surrounding environment, typically involving exchange of water overlying a sediment core. The use of continuous flow systems has been much less common for the determination of benthic exchange rates in marine systems.

There is a large variety in configuration and purpose among the continuous flow systems designed for the determination of benthic exchange rates [Pamatmat (1965), marine, O_2 ; Gallepp (1979), freshwater, PO_4 ; Bottom (1981), marine, O_2 ; Liere et al. (1982), freshwater, PO_4 ; Nishio et al. (1983), O_2 , DIN (dissolved inorganic nitrogen); Kautsky (1984), aquatic systems, O_2 ; Twinch & Peters (1984), freshwater, PO_4 ; Fowler et al. (1987), freshwater, Si, PO_4 ; Sundback & Graneli (1988), marine, O_2 , DIN, PO_4 , Si; Yamamuro & Koike (1993), marine, PN (particulate nitrogen), DIN, DON (dissolved organic nitrogen), PO_4 ; Hansen & Blackburn (1992), marine, O_2 , CO_2 , NH_4 , NO_3+NO_2 , DON; Risgaard-Petersen et al. (1994), aquatic systems, O_2 , NH_4 , NO_3 , N_2]. These continuous flow microcosm systems consist of 3 basic components: the supply reservoir(s), the microcosms, and a means of moving water through the system, typically either by gravity or a peristaltic pump. Benthic exchange rates are calculated from concentration differences between the influent and effluent lines. Concentration differences between influent and effluent lines are a function of the residence time of water over the microcosm, which is controlled by the supply rate and the volume of water in the core overlying the sediment. Fluxes are determined using the following general equation.

$$\text{Flux} = (C_E - C_I) \times \text{supply rate}$$

where C_I is the influent concentration of a parameter and C_E is its effluent concentration. Flux is then converted to an areal basis where appropriate.

There has been no extensive development of the design, operation and theory of continuous flow sys-

tems for the determination of benthic exchange rates. This paper discusses the theoretical and practical use of continuous flow systems for this purpose. Results from an experimental comparison of closed (batch) and continuous flow (open) systems are presented and discussed in light of the theoretical development. Lastly, experiments exploring the sensitivity of flux determinations to water residence time in a continuous flow system are presented.

THEORETICAL ASPECTS OF CONTINUOUS FLOW SYSTEMS

Closed (batch) systems are isolated from exchange with the surrounding environment. For processes which are dependent on environmental conditions, changes in conditions over time resulting from isolation have a proportionally negative effect on the accuracy of rates. For example, in a closed system, consumption of O_2 by the benthos results in decreased O_2 concentrations in the overlying water which may in turn result in decreased benthic consumption rates (Kemp & Boynton 1980). Thus, in a closed system, determinations of fluxes may vary over time as an artifact of the experimental isolation. The limitations of closed systems have been recognized by many researchers (Odum & Hoskin 1958, Jannasch 1974, Kemp & Boynton 1980, Kautsky 1984). In practice, these problems have been minimized with the use of short incubations or successive determinations of solute concentration. With the latter, rate determinations are discontinued when this rate change, known as feedback and evidenced as curvilinearity in concentrations over time, is detected.

In bacterial and algal culture, the problem of environmental dependency associated with closed systems has been avoided with the use of continuous culture. One type of continuous culture apparatus is the chemostat (Monod 1950, Novick & Szilard 1950), which is operated by controlling the supply rate of substrate into a vessel. A constant supply rate results in constant environmental conditions and steady state growth ensues. The essential features of a bacterial/algal chemostat are a well-stirred, constant volume culture vessel and the introduction of fresh medium at precisely controlled rates. An analogous continuous flow benthic microcosm system could minimize the problem of changing environmental conditions and result in non-varying or steady state fluxes over time. The ability to study benthic exchange processes under steady state conditions would permit cause-effect studies of factors influencing benthic exchange patterns.

The dilution rate, D , defined as the ratio of the supply rate to the culture volume, is critical to the opera-

tion of a continuous flow system (Herbert et al. 1956, Williams 1965, Ogunrombi & Dobbins 1970, Conway 1974). The reciprocal of the dilution rate, $1/D$, is the mean residence time of media within a chamber or microcosm. In bacterial and phytoplankton continuous culture, dilution rate choice is dictated in part by the growth kinetics of the species under culture. Optimal dilution rates in the benthic chemostat are similarly determined by the kinetics of the exchange process under investigation. In an algal chemostat, washout occurs at high dilution rates when the flushing rate of cells from the culture is greater than the rate of population growth. Too high a dilution rate (short residence time) in a benthic chemostat system results in its benthic analog in which the residence time of the overlying water is less than the time required for sediment-water exchanges to affect water column concentrations, resulting in influent and effluent concentrations which are statistically identical (Fig. 1). At low dilution rates (long residence time), changes in overlying water concentrations due to sediment-water exchange result in large departures from initial environmental conditions. This departure from initial conditions results in the development of feedback effects as defined above (Fig. 1). For a benthic chemostatic system, optimal dilution rates are a function of the kinetics of the exchange process and occur when solute concentration differences between influent and effluent lines are statistically separable for the analytical precision of the solute (Fig. 1; note error bars). However, dilution rates should also minimize differences in effluent and influent concentrations so that exchange rates are unaffected by deviations from ambient conditions (Fig. 1). At a constant (and optimal) dilution rate, steady state conditions in a benthic continuous flow system should result when water volume and environmental conditions are also held constant. Steady state conditions are defined as a non-varying flux with time. Under steady state, successive rate determinations should be statistically identical. Multiple determinations add statistical robustness to conclusions drawn from an experiment.

There may be an interest in determining fluxes under non-steady state conditions in a continuous flow system such as examining a response to some perturbation. Under non-steady state conditions, flux can no longer be estimated as a product of supply rate and the difference in influent and effluent concentrations. Under these conditions, it is necessary to use a mass balance approach

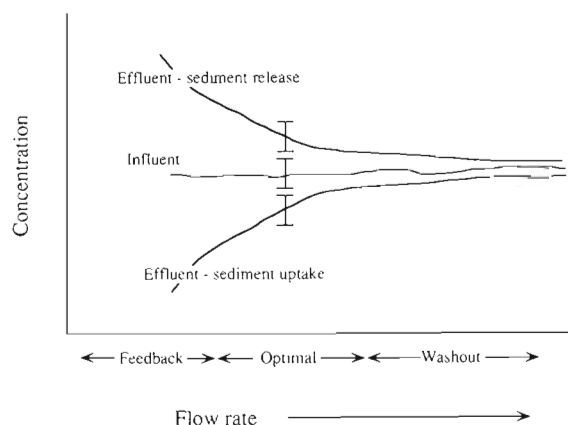


Fig. 1 Theoretical relationship between supply rate and solute concentrations in a benthic continuous flow system. Error bars represent analytical error in the determination of solute concentration

wherein the loading (amount in), loss (amount out) and production are determined over time.

METHODS

Continuous flow microcosm design and operation.

Design: The design of the continuous flow system used in this study is presented in Fig. 2. In this design, a peristaltic pump draws water from the reservoir into the experimental core and from the core to the sampling flasks. Tygon™ tubing connects the water reservoir, cores and peristaltic pump using polypropylene connectors where necessary. Outflowing water from the core is routed through a series of stopcocks and collected in flasks during sampling, while being dis-

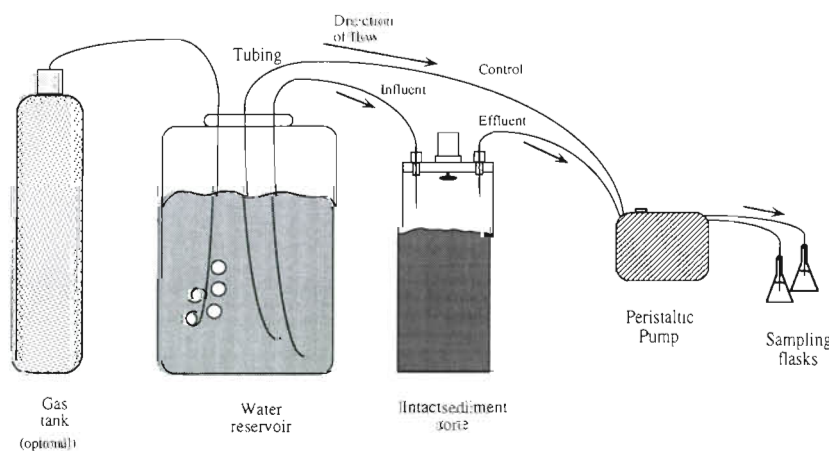


Fig. 2. Components of the continuous flow microcosm system showing connections for an individual core

carded at other times. The rate of water flow through the system is determined by the pump speed and the diameter of pump tubing. In this design, the peristaltic pump (Ismatec™ multichannel cartridge pump) operates a maximum of 16 lines (represented by a combination of experimental cores and reservoir lines) and can generate supply rates from 0.004 to 24 ml min⁻¹ using 0.19 to 2.79 mm ID tubing.

In this design, an individual microcosm (sediment core + overlying water) consists of a Lexan™ tube 15 cm in diameter and 30 cm in height (Fig. 2). The core is made water and gas tight with plexiglass disks at either end. These disks are held fast using rubber caps (bottom) and sleeves (tops) and screw clamps. Rubber (pipe) caps and sleeves are standard plumbing supply items and are available in a range of standard pipe diameters. With a typical 20 cm sediment core, the water column overlying the sediment has a volume of 1800 ml. It is kept homogeneous by stirring with an internally mounted Nalgene™ floating stir bar (3.8 cm length). The internal stir bar is driven by an external miniature magnetic stir plate mounted upside down on the top of the core. The disk at the upper end of the core has 3 ports, sealed with rubber septa, which serve as inflow, outflow, and sampling ports. Stainless steel needles with luer lock™ fittings are used in the inflow and outflow ports.

The microcosm is supplied with water collected from the study site. Thus, water column characteristics (particulates, nutrient concentrations, etc.) during the incubations mimic those in the field. For determinations of sediment metabolism and benthic nutrient regeneration rates, water is filtered through a 0.2 µm in-line capsule filter to remove suspended particulates. Changes in oxygen or nutrient concentrations between influent and effluent lines can therefore be attributed to sediment processes. Other work with this system has shown that nutrient concentrations are not altered by the collection and filtering process (Miller-Way et al. 1994). In the present design, 20 l rectangular carboys or collapsible Cubitainers™ have proven most suitable as reservoirs.

Benthic fluxes are calculated using the general equation defined above corrected for the area of sediment within the microcosm. With multiple sediment cores, influent concentrations are measured on a single line, termed the control line, which originates in the reservoir, bypasses the cores, and flows directly to the sampling flasks (Fig. 2). This control line corrects for potential tubing-induced changes in concentrations as effluent water flows to the sampling flask.

Calculations of benthic exchange rates are based on the assumption of a homogeneous water column overlying the sediment. Cores are stirred at rates which have been shown to maintain a homogeneous water

column without resuspending sediments (frequency of rotation approximately 1.5 rev s⁻¹). This is the standard procedure followed for conducting batch incubations.

Operation: Intact sediment cores are collected in the field either by diving or subcoring box cores. Cores are inserted approximately 20 cm into the sediment and removed with overlying water. Cores are capped with plexiglass disks and rubber caps, secured with screw clamps, and placed in the dark for transport. Bottom water for the reservoirs is collected in the field using a submersible pump with attached hosing or a shipboard CTD system. Field temperature and salinity are recorded. Upon return to the laboratory, the temporary caps are removed from the top of the cores and replaced with disks having inlet and outlet ports. The overlying water column is flushed with approximately 20 l of filtered, ambient water, plumbed with tubing and placed in a large Nalgene tank at ambient temperature, controlled with a circulating heat exchanger (Neslab™ CFT 25).

Sampling is initiated after steady state conditions are achieved. In practice, steady state is recognized by stability in O₂ fluxes, as typically there is no real time return on nutrient data. Steady state conditions are not immediately attained due to the perturbations arising from collection and transport procedures. Salinity dilution experiments using the design in this study have shown that >90% of the overlying water is replaced within 3 turnovers (F. Fernandez pers. comm.). In practice, the time to steady state is typically less than 2 times the residence time, or approximately 4 to 6 h. Repeated samplings are spaced in time such that a complete turnover of the water column occurs between each sampling. For example, at a typical supply rate of 10 ml min⁻¹, the turnover or residence time of the overlying water is 3 h in the core design described above. Using this design, no signs of experimental artifacts, i.e. decline in sediment oxygen consumption or nutrient fluxes, have been noted in incubations lasting more than 4 d. However, Nishio et al. (1983) showed that the growth of microorganisms on core surfaces and connecting tubes, redox changes, and the removal of surface sediments limited the duration of experiments in their flow through microcosm to approximately 30 h. Hansen & Blackburn (1992) noted no 'container' artifacts in their experiments lasting 20 d. Sundby et al. (1986) noted that the depletion of organic matter via remineralization resulted in reduced fluxes after a period of approximately 3 wk. These time scales vary among continuous flow systems as a result of different chamber configurations. In our system, bacterial growth in the connecting tubing has been noted on only 1 occasion which was thought to be due to high temperatures and the addition of a concentrated spike of ¹⁵NH₄ to the reservoir water.

Statistical analysis: Given steady state conditions, rate determinations should be statistically identical at any time during the experiment. However, the high degree of correlation of these multiple measurements made on a single core over time necessitates the use of a repeated measures design. Both the multivariate and univariate approaches to repeated measures have been used (Davidson 1972, Gurevitch & Chester 1986, Moser et al. 1990). In the latter, it is necessary to characterize the correlation structure using Mauchly's criterion (Mauchly 1940) and, when necessary, to apply published corrections for the degrees of freedom (Greenhouse & Geisser 1959, Huynh & Feldt 1970). The univariate approach (with appropriate corrections to the degrees of freedom) is favored due to problems with occasional missing data; a multivariate approach will not handle missing cells. The utility of a specific approach varies with the type of experiment. In most cases, however, treatment effects lie in the main plot of the model and results are identical using either approach.

Study site. The experiments described below were conducted at 2 different stations in the Fourleague Bay estuarine system. Fourleague Bay is a shallow (mean depth of 1.5 m), river-dominated estuary in south-central Louisiana (USA) characterized by high suspended sediment and nutrient loads (Madden et al. 1988). The upper bay station, Carencro Bayou, is of low salinity (averaging <1‰ annually); the lower bay station, Oyster Bayou, is more typically estuarine with salinities (5 to 25‰) varying seasonally with the discharge of the Atchafalaya River. Infaunal benthic populations at these stations are low (Miller-Way unpubl.) presumably due to frequent wind-induced sediment resuspension. Sediments at both stations consist primarily of silt and clay (>90%), have a high organic carbon content (ca 6% of dry mass), and are well oxidized (mean Eh at 1 cm depth was 261 mV for cores used in this experiment). Frequent resuspension coupled with predominantly silt/clay-sized particles create a very fluid sediment.

Comparison of closed and continuous flow fluxes. Benthic nutrient fluxes and sediment oxygen consumption were determined using closed system (batch) methodology and compared to those measured using the continuous flow microcosm during experiments conducted in April 1991. Experiments were conducted at both Fourleague Bay stations described above. Temperatures in April were 25 and 23°C, and salinities were 0 and 18‰ at Carencro and Oyster Bayous, respectively.

At each station, 12 cores were collected, 6 being allocated to each method. Exchange rates were determined for oxygen, NH_4 , NO_3 , NO_2 , PO_4 and $\text{Si}(\text{OH})_4$. Three determinations of fluxes were made for each

replicate core in the continuous flow system. Four determinations of concentrations (3 flux estimates) were made for the batch cores. Core design, treatment and sampling frequency were identical for batch and continuous flow incubations; inlet and outlet ports were simply plugged for the batch cores. Water removed from the batch cores for nutrient determinations (approximately 30 ml of a total volume of 1800 ml) was replaced with filtered ambient water after each sampling. A supply rate of 10 ml min^{-1} , corresponding to a water residence time of 3 h, was chosen for continuous flow microcosms on the basis of previous experiments at these stations. The duration of each experiment was approximately 10 h. Cores were incubated in the dark to minimize variability caused by benthic autotrophic processes. Oxygen concentrations were measured using an Orbisphere oxygen meter (polarographic electrode); the sensor was inserted into the core's sampling port. Ammonium, NO_3+NO_2 , PO_4 , and $\text{Si}(\text{OH})_4$ concentrations were measured using standard colorimetric techniques (Strickland & Parsons 1972) on an Alpkem RF/A II autoanalyzer. By convention, sediment O_2 consumption is expressed as a positive value while sediment nutrient uptake is expressed as a negative value.

Sensitivity of fluxes to water residence times. The determination of steady state conditions in a continuous flow system is dependent on the rate kinetics of the process under investigation. It was therefore of interest to determine the sensitivity of benthic metabolism and nutrient fluxes to dilution rate or water residence time.

The variation of sediment oxygen consumption with dilution rate was determined on 2 occasions. Initial determinations were conducted in January 1991 using 5 replicate sediment-water microcosms collected from Carencro Bayou. Single flux measurements were made at 5 distinct supply rates ranging from 4 to 20 ml min^{-1} and measured in ascending order, but including a return to the lowest supply rate at the end of the experiment. These supply rates correspond to residence times of 7.5 h (4 ml min^{-1}) to 1.5 h (20 ml min^{-1}). One and one-half complete replacements of the water column overlying each sediment core were allowed before measurements were made, although O_2 data indicated that steady state occurred within one-half of a turnover (water residence time). This experiment was repeated in September 1991 with 6 replicate sediment cores from the same station using the same range of supply rates (residence times) tested in January. Single flux determinations were again made for each supply rate, however, supply rates were tested in random order. Oxygen concentrations were measured using an Orbisphere oxygen meter.

To investigate the effects of supply rate (residence time) on benthic nutrient flux, exchange rates were determined for the September experiment described above. Nutrient [NH_4 , NO_3 , NO_2 , PO_4 and Si(OH)_4] concentrations were determined using the protocol cited above.

RESULTS

Comparison of closed and continuous flow fluxes

Sediment oxygen consumption was significantly lower in the batch cores for both stations (Table 1). Fluxes were not significantly different between stations (Table 1) but showed significant decreases with time regardless of method (Tables 2 & 3). Benthic nutrient fluxes determined using the continuous flow system were significantly higher for NH_4 (Oyster Bayou), NO_2 (Oyster Bayou), and PO_4 (Oyster Bayou) and Si(OH)_4 (both stations) (Fig. 3). Although all cores were taken from the same area, variation in sediment chlorophyll, benthic infauna and other sediment characteristics among cores contributed to the high coefficient of variation among replicate cores.

There were significant interactions between time and method for most nutrients (Table 3), indicating that batch and continuous flow incubations behave differently with time. Nitrate fluxes were generally consistent with time for the continuous flow method while decreasing with time in the batch cores. There was no

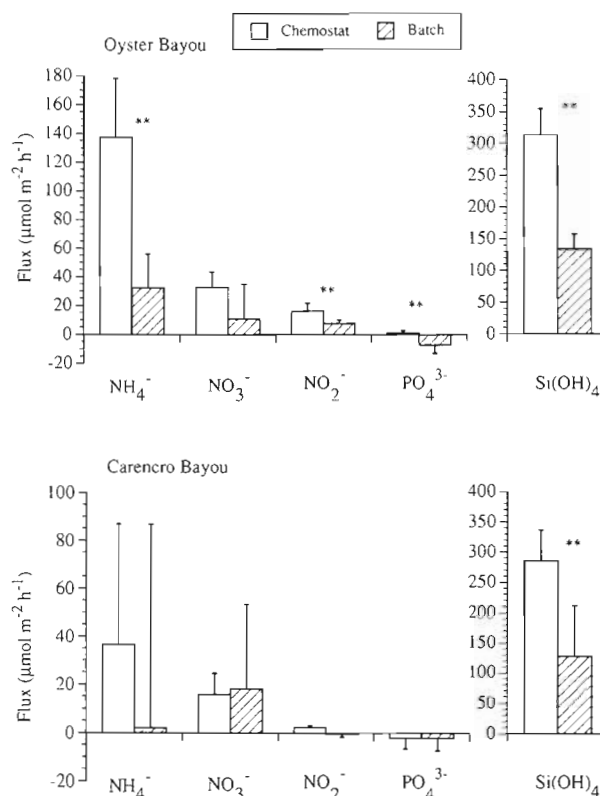


Fig. 3. Sediment fluxes of nutrients at 2 stations as measured using continuous flow and batch techniques. Values represent means for 6 cores at 3 samplings; error bars represent standard deviations. Asterisks denote significance at the 0.05 level

Table 1 Sediment oxygen consumption rates and the main plot of the linear model used to test for differences among rates determined using batch and chemostat methodologies. Station by method interactions were pooled according to the criteria of Bancroft & Chien-Pai (1983). Average initial O_2 concentrations ($n = 6$) in the batch and chemostat cores are indicated by their respective stations

Station	Oxygen consumption ($\text{g m}^{-2} \text{d}^{-1}$)		Initial concentrations (mg l^{-1})	
	Batch	Chemostat	Batch	Chemostat
Oyster Bayou				
\bar{x}	0.61	0.90	7.82	7.45
(SD)	(0.14)	(0.11)		
Carencro Bayou				
\bar{x}	0.59	0.86	7.69	8.05
(SD)	(0.11)	(0.17)		
Source model				
Source	df	F	p	
Station	1	3.97	0.0595	
Method	1	197.69	0.0001	
Core (Station, Method)	21	0.81	0.6906	

apparent pattern with time for PO_4 fluxes. The decrease in Si(OH)_4 flux with time was similar for both techniques (Table 2).

Sensitivity of fluxes to water residence times

Rates of sediment oxygen consumption in January were not significantly different for supply rates ranging from 8 to 16 ml min^{-1} (Fig. 4), corresponding to residence times of 3.8 to 1.8 h. Significantly lower consumption rates were measured at 4 ml min^{-1} and significantly higher rates at 20 ml min^{-1} ($F = 16.54$, $df = 4, 25$). Initial and final rates of sediment oxygen consumption at 4 ml min^{-1} were not significantly different from each other ($F = 0.16$, $df = 1, 8$). Measurements made in September indicate a similar pattern, despite the difference in the order of supply rate determinations (Fig. 4). Supply rate had a significant effect on sediment oxygen consumption ($F = 69.98$, $df = 4, 25$): rates were significantly lower at 4 ml min^{-1} and higher at 20 ml min^{-1} ; rates at 8, 12 and 16 ml min^{-1} were not statistically different. Temperature differences (24 vs 31°C for January and September, re-

Table 2. Mean flux rates over time determined using the chemostat (Chem.) and batch methodologies. Approximate time between samplings was 3 h. nd: no data

Time	N	NH ₄		NO ₃		NO ₂		PO ₄		Si(OH) ₄		O ₂		
		(μmol m ⁻² h ⁻¹)		(μmol m ⁻² h ⁻¹)		(μmol m ⁻² h ⁻¹)		(μmol m ⁻² h ⁻¹)		(μmol m ⁻² h ⁻¹)		(g m ⁻² d ⁻¹)		
		Chem.	Batch	Chem.	Batch	Chem.	Batch	Chem.	Batch	Chem.	Batch	Chem.	Batch	
Oyster Bayou														
T ₁	\bar{x}	6	182.08	36.79	41.96	27.01	19.66	8.86	1.21	-12.60	386.65	233.60	1.05	0.74
	(SD)		(34.83)	(20.55)	(11.33)	(5.94)	(6.37)	(2.00)	(2.43)	(5.22)	(65.97)	(34.18)	(0.05)	(0.08)
T ₂	\bar{x}	6	122.20	26.70	25.36	26.14	15.24	8.74	-0.31	-3.50	275.88	178.24	0.87	0.69
	(SD)		(19.64)	(22.53)	(8.92)	(4.48)	(4.93)	(2.30)	(1.56)	(1.69)	(43.99)	(23.95)	(0.02)	(0.07)
T ₁	\bar{x}	6	107.34	33.84	30.71	-21.00	13.38	5.87	1.97	-5.44	278.52	-9.89	0.79	0.45
	(SD)		(18.87)	(29.64)	(4.62)	(9.73)	(4.22)	(2.07)	(1.07)	(5.22)	(23.99)	(33.91)	(0.02)	(0.05)
Carencro Bayou														
T ₁	\bar{x}	6	62.35	-60.30	11.71	25.65	2.84	-1.20	-1.57	-6.45	416.26	141.97	1.01	0.64
	(SD)		(70.32)	(112.66)	(9.92)	(31.26)	(0.75)	(0.44)	(4.46)	(6.03)	(91.13)	(136.97)	(0.12)	(0.04)
T ₂	\bar{x}	6	35.40	20.72	18.20	18.57	1.37	-0.61	-2.21	-1.49	398.68	148.59	0.90	0.54
	(SD)		(38.96)	(52.06)	(9.11)	(40.87)	(1.06)	(1.24)	(4.30)	(3.10)	(60.33)	(144.82)	(0.07)	(0.12)
T ₃	\bar{x}	6	11.66	45.59	17.18	10.15	2.09	0.13	-2.42	1.70	nd	89.67	0.67	0.57
	(SD)		(25.32)	(40.77)	(7.22)	(38.13)	(0.72)	(1.54)	(2.57)	(3.04)		(80.48)	(0.08)	(0.13)

spectively) between the 2 periods account for the relative differences in oxygen consumption rates.

Nutrient fluxes measured at a supply rate of 8 ml min⁻¹ were significantly different from those measured at all other supply rates (Table 4). This was the first supply rate tested; sampling occurred 5.3 h after the beginning of the experiment (1.5 complete turnovers of the water column overlying the sediment core). These fluxes were most likely determined

Table 3. Tests of significance for all nutrients for subplot of repeated measures model. Tests for each nutrient were run individually and all tests were made using the Huynh-Feldt adjustment to the degrees of freedom (see text). ns: not significant ($p > 0.05$); s: significant ($p < 0.05$)

Source	NH ₄ ⁺	NO ₃ ⁻	NO ₂ ⁻	PO ₄ ³⁻	Si(OH) ₄	O ₂
Time	ns	ns	s	s	s	s
Time × Station	ns	s	s	ns	ns	ns
Time × Method	s	s	s	s	ns	ns
Time × Station × Method	ns	ns	ns	ns	s	s

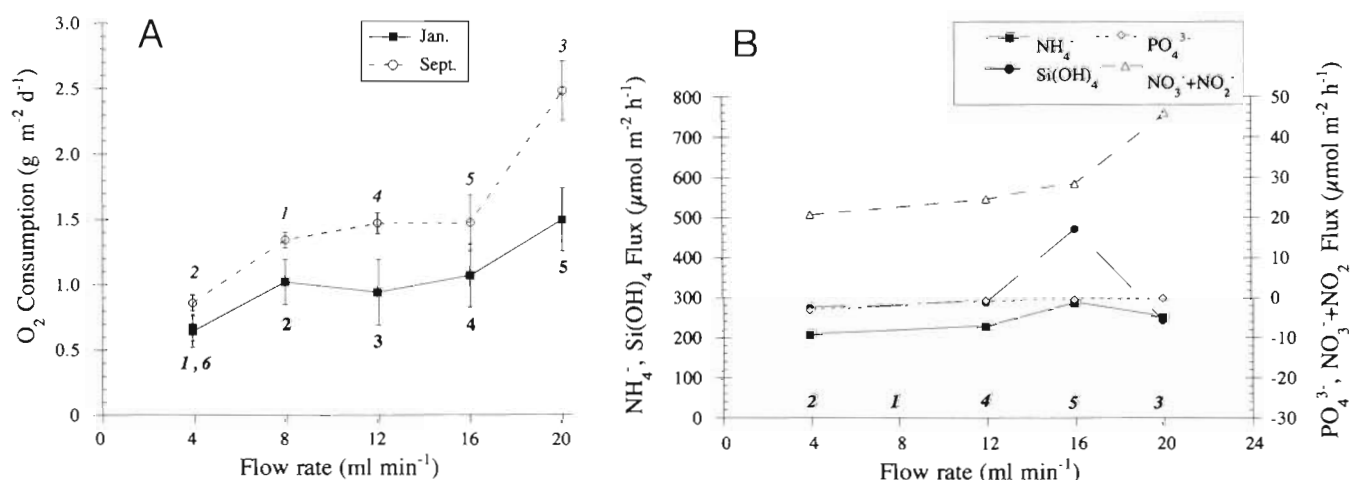


Fig. 4. (A) Oxygen consumption and (B) nutrient flux versus supply rate (residence time) in a continuous flow system. Numbers above the data point or axis indicate the sequence of determinations. Values are means for 6 cores; error bars represent standard deviations

Table 4. Fluxes of dissolved nutrients at different flow rates. Rates with the same letter for each nutrient were not significantly different using Scheffé's range tests ($p > 0.05$). *Omitted from statistical analysis

Flow rate (ml min ⁻¹)	Residence time (h)	Sequence	Nutrient flux (μmol m ⁻² h ⁻¹)							
			NH ₄ ⁺		NO ₃ ⁻ +NO ₂ ⁻		PO ₄ ³⁻		Si(OH) ₄	
4	7.5	2	\bar{x} (SD)	209.79 (74.73)	A	21.10 (4.44)	A	-2.88 (0.28)	A	276.67 (49.59)
8	3.75	1	\bar{x} (SD)	586.21 (93.28)	*	2.27 (6.55)	*	-15.34 (1.71)	*	685.87 (95.13)
12	2.5	4	\bar{x} (SD)	230.92 (96.69)	A	24.86 (5.56)	A	-0.43 (0.28)	C	291.33 (73.51)
16	1.88	5	\bar{x} (SD)	288.88 (157.96)	A	28.71 (6.18)	A	-1.71 (0.79)	B	407.47 (48.97)
20	1.5	3	\bar{x} (SD)	252.94 (121.93)	A	45.90 (8.16)	B	0.13 (1.62)	C	253.33 (86.41)

prior to the attainment of steady state conditions. Omitting these initial measurements from the statistical analysis showed that fluxes varied significantly with supply rate for NO₃+NO₂ and PO₄ (Table 4). However, there was no consistent pattern of PO₄ fluxes with

changes in supply rate; reasons for this are illustrated in Fig. 5 and discussed below. Fluxes of NO₃+NO₂ were significantly greater at 20 ml min⁻¹; possible reasons for this are discussed in context with Fig. 5. It is interesting to note that the observed initial disturbance resulted in increased NH₄ and Si(OH)₄ release, increased PO₄ uptake and decreased NO₃+NO₂ release (Table 4) and that rates of sediment oxygen consumption did not exhibit this disturbance artifact. Differences among nutrients will be addressed below.

Fig. 5 demonstrates the effect of changing residence time (supply rate) on influent and effluent nutrient concentrations during the September experiment. Effluent concentrations follow the expected pattern described by Fig. 2; as supply rates increase, differences between influent and effluent concentrations decrease. Influent and effluent PO₄ concentrations were statistically indistinguishable at supply rates of 12 ml min⁻¹ and greater, illustrating the washout effect at these supply rates (Fig. 5). Effluent concentrations of NO₃+NO₂ were higher than expected at 20 ml min⁻¹ (supply rate) resulting in significantly higher fluxes. This pattern was also observed for O₂. Both unexpected values follow determinations at the lowest supply rate when differences between influent and effluent concentrations were maximal. It is likely, therefore, that the turnover of the overlying water allowed between samplings was not sufficient to regain steady state for these 2 analytes. This result illustrates the differing sensitivities of the nutrients measured to residence time.

DISCUSSION

Validity of comparison

It has long been recognized that stirring of either *in situ* chambers or microcosms increases sediment

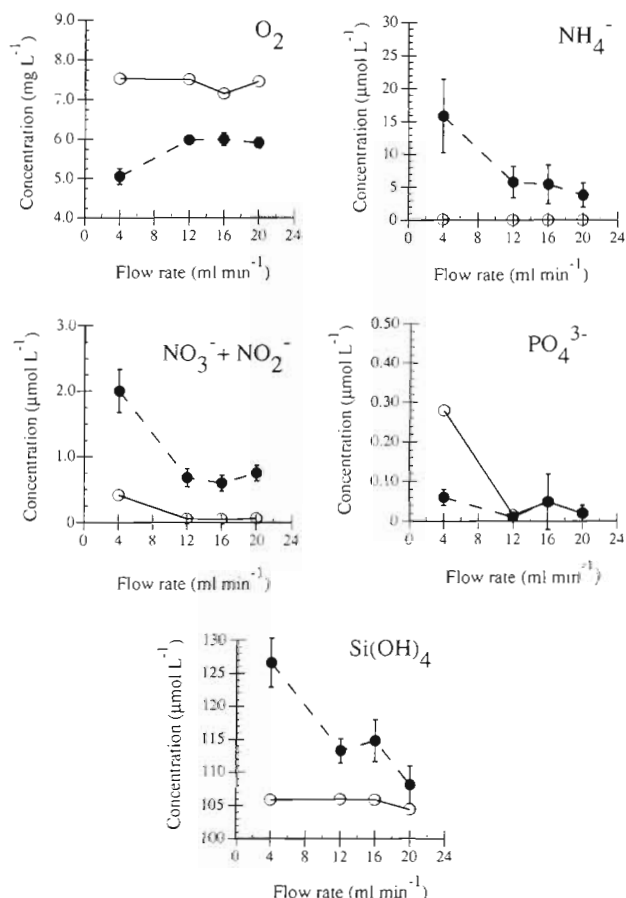


Fig. 5. Influent and effluent concentrations vs supply rate for each solute. Open points denote influent concentrations; closed points denote effluent concentrations. Values are means for 6 cores; error bars represent standard deviations

exchange rates (Carey 1967, Davies 1975, Boynton et al. 1981). Thus, across design comparisons may be erroneous because different stirring devices may create different flow patterns within a chamber or core and result in different effective diffusion coefficients (Santschi et al. 1983, Jørgensen & Revsbech 1985).

The observed differences between the 2 techniques were not due to additional mixing resulting from the movement of water through the continuous flow microcosms. In the continuous flow system, influent and effluent lines discharge from small diameter (18 gauge) needles approximately 7 cm above the sediment-water interface and on opposite sides of the stirrer. Observations with dye showed that the stirrers quickly entrain influent water. The effects of the stirrer on flow patterns within the core are much greater than

that contributed by the influent and effluent lines, and stirring therefore controls hydrodynamics within the microcosms. Differences between solute response to changing supply rate (using the same experimental design) lend support to this conclusion (see below). Stirring, and therefore hydrodynamic conditions at the sediment-water interface, was similar for both techniques.

Patterns of benthic flux

For *in situ* or laboratory microcosms (i.e. assuming negligible deposition, compaction, and bioturbation), sediment-water exchange rates of oxygen and nutrients follow Fick's first law of diffusion.

$$J = -\phi D_s (\delta C / \delta z)_{z=0}$$

where ϕ is sediment porosity, D_s is the whole sediment molecular diffusion coefficient, and $(\delta C / \delta z)_{z=0}$ is the concentration gradient across the sediment-water interface (z positive downward) (Berner 1980). In these experiments, initial terms in the equation were similar among supply rates (residence times) or treatments (batch vs continuous flow). Therefore, changes in concentration gradients among supply rates or treatments produced the observed results. These changes produced 3 identifiable patterns of sediment-water exchange which can be termed feedback, linear, and washout responses. The results of the 2 experiments described above (batch vs continuous flow, sensitivity to residence time) are integrated by regarding batch incubations as one endpoint of a continuum of supply rates, that is, as a continuous flow microcosm with a zero supply rate (i.e. infinite residence time).

Feedback response

Nitrate fluxes at Oyster Bayou demonstrated a feedback response (Fig. 6). At Oyster Bayou, NO_3^- concentration in the batch cores initially increased linearly with time (Fig. 6A). Fluxes calculated from the slope of this line are similar for both time intervals ($T_0 - T_1$, $T_1 - T_2$) (Fig. 6B). However, by the last sampling, a decrease in overlying water concentration was observed which most likely resulted from a change in the concentration gradient between sediment and overlying water. The calculated flux for this time interval ($T_2 - T_3$) is therefore opposite in direction from the previous time intervals. Using the regression approach for flux calculations in batch incubations (concentration vs time relationship), the departure from linearity results in a decreased flux (lower slope) compared to that based on the first 2 samplings. In contrast, fluxes in the

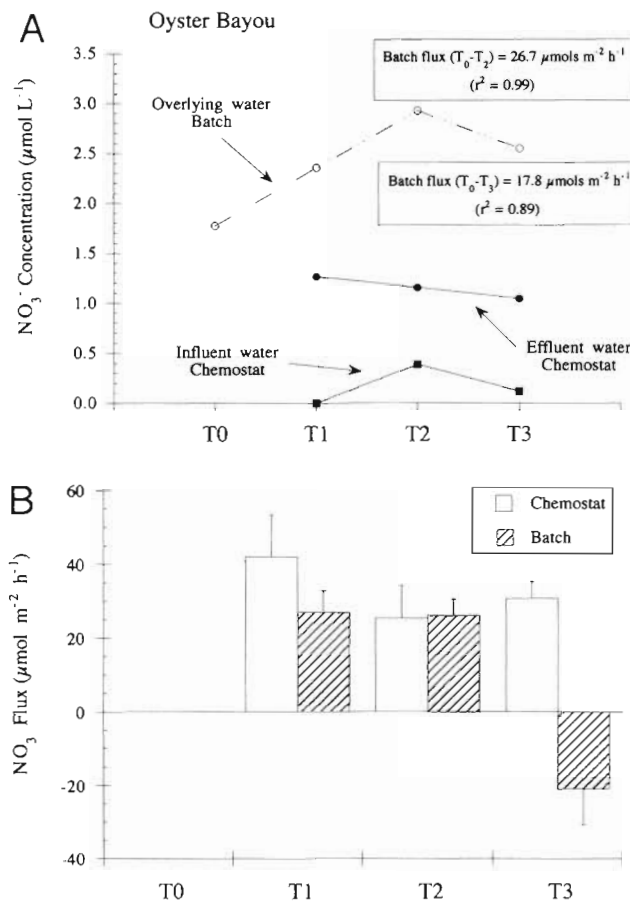


Fig. 6. (A) Nitrate concentration over time at Oyster Bayou (Louisiana, USA) in batch and continuous flow microcosms and (B) the corresponding fluxes. Note the nonlinear change in overlying water concentration in the batch cores. Fluxes given in the boxes are those calculated using the regression approach for the specified time interval of the incubation. Note that the rate for the first 2 time intervals is greater than that for all samplings as a result of the nonlinearity in concentrations. Error bars as in Fig. 3

continuous flow microcosms were higher and relatively stable with time as influent and effluent concentrations did not vary with time (Fig. 6).

Linear response

Nitrate flux at Carencro Bayou typified a linear response. Overlying water concentrations increased linearly ($r^2 = 0.95$) with time for the batch cores, while influent and effluent concentrations in the continuous flow cores did not change significantly with time (Fig. 7A). Concentration differences between successive samplings for the batch methods were stable with time and therefore resulted in fluxes which were stable with time (Fig. 7B). Similarly, fluxes for the continuous

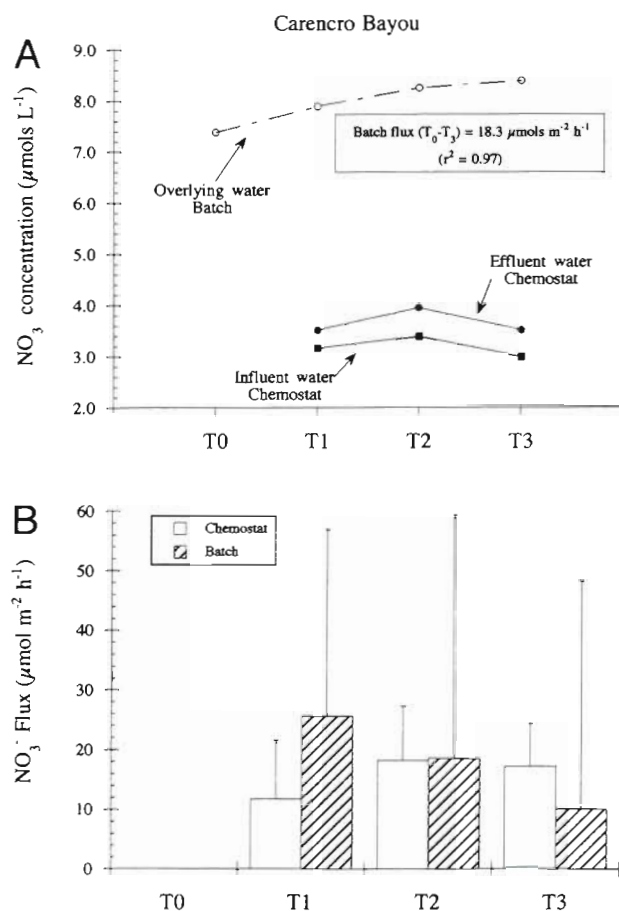


Fig. 7 (A) Nitrate concentration over time at Carencro Bayou (Louisiana, USA) in batch and continuous flow cores and (B) the corresponding fluxes. Note the linear increase in overlying water NO_3^- concentration in the batch cores and the stability of the corresponding fluxes with time. Note the similarity of the flux calculated using the regression approach (box) to those calculated for the continuous flow approach and for individual time intervals in batch incubations (B). Error bars as in Fig. 3

flow cores were stable with time, reflecting concentration differences which were also stable with time (Fig. 7). In a linear response, fluxes are stable with time. Thus, no significant differences were observed between techniques.

The sole difference between linear and feedback responses is that of time. Given sufficient time in a closed system (batch core), the exchange of nutrients/oxygen across the sediment-water interface will change (decrease) concentration gradients and result in a feedback effect.

Washout response

Phosphate fluxes at Carencro Bayou typified a washout response (Fig. 8). For example, although PO_4 fluxes determined with the continuous flow method appeared stable with time (Fig. 8B), they do not represent realistic rate estimates. Examination of PO_4 concentrations for the continuous flow cores show that washout occurred as influent and effluent values were not statistically different (Fig. 8A). In a washout response, ion exchange across the sediment-water interface is insufficient to allow for significant changes in the overlying water at the experimental supply rate. This type of response can be recognized by fluxes which are not significantly different from 0. In order to distinguish a washout response from an analytically real flux of 0, it is necessary to alter the supply rate and redetermine the flux.

Observed differences between techniques

The general pattern of results indicated that nutrients with high rates of flux showed pronounced differences between batch and continuous flow techniques. As exchange across the sediment-water interface occurred, changes in overlying water concentrations reduced gradients which in turn resulted in larger departures from initial conditions and large differences between treatments. In contrast, nutrients with lower rates of exchange did not alter initial concentration gradients as rapidly and differences between techniques were less pronounced. The severity of these feedback effects varied with nutrient (see below) and with station and were in concordance with the degree to which ambient concentration gradients were altered. Emerson et al. (1984) also noted differences among nutrient flux which were explained by the strength of the respective sediment-water concentration gradient.

Relative interstitial pool sizes play a critical role in determining the pattern of response via their effects on

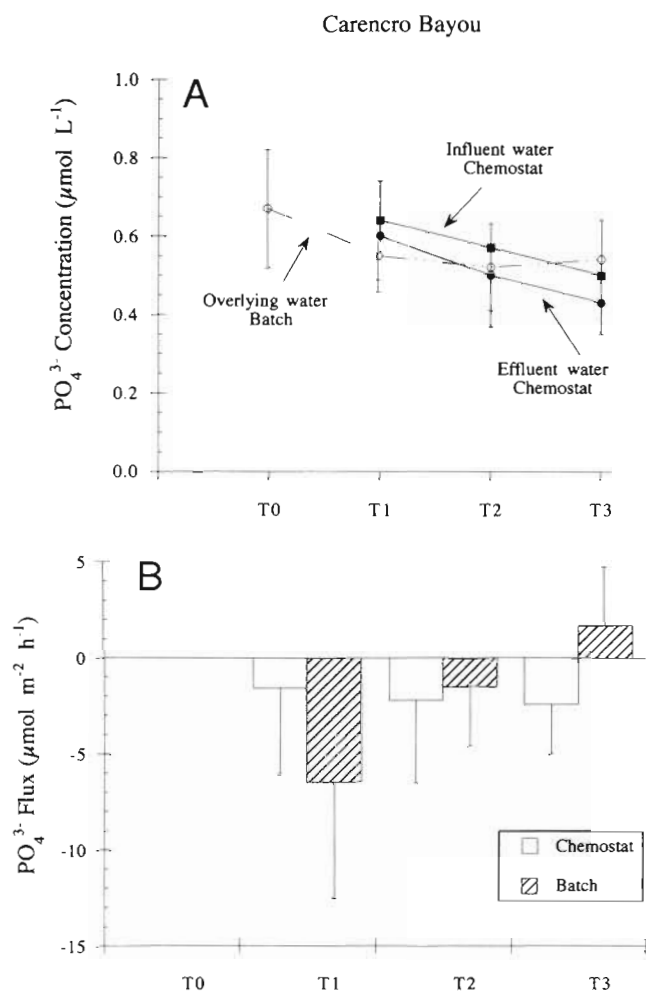


Fig. 8. (A) Phosphate concentration over time at Carencro Bayou in batch and continuous flow cores and (B) the corresponding fluxes. Note the absence of statistically different concentrations for the influent and effluent lines of the continuous flow cores. Error bars as in Fig. 3

sediment-water concentration gradients. A large nutrient pool can support a given flux without resulting in significant changes in concentration gradients. For example, NH_4 pore water concentrations are typically high (95 to 654 μM , 0 to 2 cm depth) at Oyster Bayou (Twilley unpubl. data). Using an average value for pore water concentrations (300 μM), with average overlying water concentrations (1 μM) and fluxes (100 $\mu\text{mol m}^{-2} \text{h}^{-1}$) observed during the experiment (and assuming no NH_4 production during this time), at the end of 3 h (residence time in this experiment), overlying water concentrations have increased to 4 μM while pore water concentrations have decreased to approximately 285 μM . These changes reflect an approximate 6% change in the initial concentration gradient. In contrast, initial concentration gradients of

NO_3 , which typically has low pore water concentrations at this site (0.1 to 3.4 μM ; Twilley unpubl. data), are dramatically altered during an incubation. Again, assuming average pore water (2 μM) and overlying water concentrations and fluxes as observed in the experiment (1.5 μM and 25 $\mu\text{mol m}^{-2} \text{h}^{-1}$, respectively), during a 3 h incubation, the gradient between sediment and overlying water concentrations is eliminated. Release of NO_3 by the sediment causes a depletion of pore water NO_3 (assuming no further production during this time), while the overlying water concentration increases to approximately 2.3 μM .

Observed differences among solutes

The observed variable response of oxygen consumption and nutrient flux to residence time implies that the kinetics of benthic exchange differ among solutes. The kinetics of regeneration and exchange are governed by different biological and physical processes. As these processes differ for different solutes, each responded differently to variation in residence time. For example, concentration gradients of $\text{Si}(\text{OH})_4$ are controlled primarily by dissolution rates (through effects on pore water concentrations). In contrast, NH_4 concentration gradients reflect heterotrophic activity levels. Alternatively, pore water and overlying water concentrations of PO_4 appear to be held in an adsorption equilibrium (Sundby et al. 1986). Thus, it is not surprising that solutes responded differently to changes in residence time. However, these differences indicate that the optimal residence time (supply rate; minimum difference between influent and effluent concentrations which are statistically distinct) is different for each solute and that supply rates must be selected for the rate process under study.

Advantages of continuous flow methodology

Continuous flow systems have many distinct advantages for the study of benthic-pelagic coupling. The physical, chemical and biological nature of the sedimentary matrix is impossible to replicate in the laboratory, yet it is this structure which is paramount in controlling many benthic exchange processes. A microcosm system using intact sediment cores collected immediately prior to the experiment permits minimal disruption of sediment layering, particle size distribution, chemical gradients, and biological communities. Water for the supply reservoirs collected concurrently enables the close replication of the *in situ* sediment-water system. Continuous flow methodology permits the maintenance of initial experimental condi-

tions for the duration of the experiment. Batch methodology is most problematic when large changes occur in ambient concentration gradients. This occurs when nutrients, or more generally any parameter, have a high flux or in experiments which are of long duration. In addition, multiple determinations of rates permit statistical rigor that can only be achieved in batch incubations by the addition of individual cores, a labor intensive process.

The modular nature of the continuous flow system described herein facilitates experimental manipulations designed to directly study mechanisms controlling benthic-pelagic coupling, a technique increasingly applied in many disciplines of ecology (Pielou 1969, Peterson 1980, Hairston 1989, Lubchenco & Real 1991). Discrete system components allow the manipulation of many environmental parameters including water column characteristics, some aspects of the benthic environment, and hydrodynamic conditions at the sediment-water interface. Changes in benthic fluxes under hypoxia have been studied by purging the supply reservoirs with nitrogen or argon gas (Miller-Way & Twilley 1993, Fernandez 1995). Simultaneous estimates of direct and coupled denitrification rates have been made by amending separate supply reservoirs with $^{15}\text{NO}_3$ and $^{15}\text{NH}_4$ and measuring the production of nitrogen gas (N_2 and N_2O) (Twilley unpubl.). The role of benthic macrofauna in benthic-pelagic coupling has also been addressed using a series of manipulated (added macrofauna) and ambient microcosms with the macrofaunal contribution assessed by subtraction (Miller-Way 1994).

There are a number of techniques available for the determination of benthic-pelagic exchange rates. Each method has advantages and disadvantages which must be considered in light of the objectives and constraints of the study (location, logistics, funding, etc.). The primary advantage of closed system techniques is their simplicity of design and operation. The major disadvantages include potential temporal constraints due to feedback effects and a limited resultant data set in an environment in which high levels of variation are the norm (Gallepp 1979, Nixon et al. 1980, Fisher et al. 1982, Kelderman 1984). The primary disadvantage of open (continuous flow) systems is their labor-intensive operation. In some circumstances, however, the advantages of increased statistical rigor, long experimental duration and manipulative capability outweigh this disadvantage.

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