



Padilla Bay

National Estuarine Research Reserve

Reprint Series No. 26
Reprinted December 1997

**NITROGEN EXCHANGE BETWEEN THE SEDIMENTS
AND WATER COLUMN IN VEGETATED AND
NON-VEGETATED SITES IN PADILLA BAY, WASHINGTON**

Nicolle R. Rutherford

March 1997

Publication No. 97-110

The Padilla Bay National Estuarine Research Reserve is one of the reserves in the National Estuarine Research Reserve System. One of the purposes of the Reserve is to facilitate research and monitoring at Padilla Bay to provide information for the conservation and management of the nation's estuaries, in particular greater Puget Sound and other estuaries in the Pacific Northwest. The Padilla Bay National Estuarine Research Reserve assists the dissemination of this information from research and monitoring by publishing a Reprint Series and a Technical Report Series.

The **Reprint Series** includes research grant reports, out of print agency reports and student reports dealing with the Padilla Bay estuary. Reports are reprinted without revision or editing. Final reports for research grants and Masters Theses should be treated as unpublished data and should not be cited without permission of the author(s).

The **Technical Report Series** includes articles, reports of research projects, data reports, bibliographies and reviews dealing with the Padilla Bay estuary.

Communications concerning receipt or exchange of Technical Reports or Reprints or submission of manuscripts should be directed to the Research Coordinator at Padilla Bay National Estuarine Research Reserve. Communications concerning the content of reports and reprints should be directed to the author(s).

Padilla Bay National Estuarine Research Reserve
10441 Bayview-Edison Road
Mount Vernon WA 98273-9668
(360)428-1558

Padilla Bay National Estuarine Research Reserve is managed by the Shorelands and Environmental Assistance Program, Washington State Department of Ecology, in cooperation with the Estuarine Reserves Division, National Oceanic and Atmospheric Administration (NOAA), U.S. Department of Commerce. The preparation of this document was financially aided through a grant to the Washington State Department of Ecology with funds obtained from NOAA/Office of Ocean and Coastal Resource Management, and appropriated for Section 306 or 315 of the Coastal Zone Management Act of 1972, as amended.



**NITROGEN EXCHANGE BETWEEN THE SEDIMENTS AND WATER
COLUMN IN VEGETATED AND NON-VEGETATED SITES IN
PADILLA BAY, WASHINGTON**

A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Nicolle R. Rutherford

March 1997

Bibliographic citation: Rutherford, Nicolle R. 1997. Nitrogen exchange between the sediments and water column in vegetated and non-vegetated sites in Padilla Bay, Washington. Master's Thesis. Western Washington University, Bellingham, Washington. 40 pp. Padilla Bay National Estuarine Research Reserve Reprint No. 26, Reprinted December, 1997.

This is a reprint of the Washington State Department of Ecology. The views expressed herein are those of the author and do not necessarily reflect the views of NOAA, the Washington State Department of Ecology or any of their subagencies. Copies of this reprint are available from the Padilla Bay National Estuarine Research Reserve.

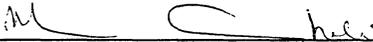
The Washington State Department of Ecology is an Equal Opportunity and Affirmative Action employer. If you have special accommodation needs, please contact the Padilla Bay Reserve at (360)428-1558 or (360)757-1549.

**NITROGEN EXCHANGE BETWEEN THE SEDIMENTS AND WATER
COLUMN IN VEGETATED AND NON-VEGETATED SITES
IN PADILLA BAY, WASHINGTON.**

BY

NICOLLE R. RUTHERFORD

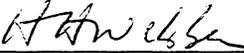
Accepted in Partial Completion
of the Requirements for the Degree
Master of Science


Moheb A. Ghali, Dean of the Graduate School

ADVISORY COMMITTEE


Chair, Emily R. Peele


David E. Schneider


Herbert H. Webber

MASTER'S THESIS

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Western Washington University, I agree that the Library shall make its copies freely available for inspection. I further agree that copying of this thesis in whole or in part is allowable only for scholarly purposes. It is understood, however, that any copying or publication of this thesis for commercial purposes, or for financial gain, shall not be allowed without my written permission.

Signature Nicole Rutherford

Date 3.10.97

NITROGEN EXCHANGE BETWEEN THE SEDIMENTS AND WATER COLUMN
IN VEGETATED AND NON-VEGETATED SITES IN PADILLA
BAY, WASHINGTON

by
Nicolle R. Rutherford

ABSTRACT. Nitrogen dynamics between the sediments and water column were compared in cores from vegetated and non-vegetated sites in Padilla Bay, Washington. Sediment cores were incubated, and sacrificed at 0, 6, 12, 24 and 48 hour time intervals to determine changes in ammonium, nitrite+nitrate, and particulate nitrogen concentrations. Ammonium and nitrite+nitrate concentrations were significantly higher in the porewaters than in the water column at both sites. Particulate nitrogen concentrations in the sediments of cores from the vegetated site were significantly higher than those in cores from the non-vegetated site. Over the 48-hour incubation, water column concentrations of ammonium and nitrite+nitrate did not vary significantly in cores from either site. However, in the porewaters, there was a significant increase in ammonium concentrations in cores from the vegetated site, and a significant increase in nitrite+nitrate in cores from the non-vegetated site. These results indicate that the nitrogen transformations within the sediments of the vegetated and non-vegetated sites are different. At the vegetated site, the accumulation of ammonium suggests extensive ammonification, while the accumulation of nitrite+nitrate at the non-vegetated site may be due to nitrification. At both sites, the sediments are acting as a source of nitrogen. However, the role of the sediments and the transformations taking place within them may change depending on the oxidation state of the sediments, the organic loading and distribution, the season, the growth stage of the macrophytes, and the abundance of benthic algae.

ACKNOWLEDGEMENTS

I gratefully thank the many people who contributed to the successful completion of this project. My committee members provided sound advice and support. Brian Bingham tolerated many statistical questions and encouraged my exploration of statistics. Dennis Bohrer and Doug Doolittle allowed me to explore the biology stockroom and gather miscellaneous equipment that proved to be invaluable in my field sampling. Gene McKeen of Shannon Point Marine Center provided support and advice, and sacrificed personal time to help me obtain my cores. Jim Murray of the University of Washington graciously allowed me access to the CHN elemental analyzer in his lab, while Jim Postel helped me with the nuts and bolts of running it. Jason Lehto offered much needed moral support, as well as assistance in the field and laboratory. Heather Nyberg spent many hours with the mortar and pestle grinding sediment samples for the elemental analysis. Finally, I thank the Padilla Bay Foundation, Shell Oil, the biology department and Shannon Point Marine Center for financial support of this thesis.

TABLE OF CONTENTS

	Page
Abstract	iv
Acknowledgments	v
List of Figures	vii
List of Tables	viii
Introduction	1
Methods	5
Site description	5
Site characterization	5
Sampling procedures	7
Chemical analysis of water, porewater and sediments	9
Experimental design and data analysis	10
Results	12
Laboratory incubation	16
Effect of NH_4^+ addition on NH_4^+ , $\text{NO}_2^- + \text{NO}_3^-$, and particulate nitrogen concentrations	20
Discussion	22
Nitrogen transformations and cycling	23
Benthic-pelagic coupling	28
Conclusion	29
Literature cited	31
Appendix A	37
Appendix B	39

LIST OF FIGURES

	Page
Figure 1. Map of Washington State showing location of Padilla Bay. The study site is indicated by an arrow on the right panel.	6
Figure 2. Percent organic matter (OM) and percent water (H ₂ O) in the sediments from the vegetated and non-vegetated sites in Padilla Bay, Washington. Mean (\pm SE) of five samples.	13
Figure 3. Grain size distribution at the vegetated and non-vegetated sites. Mean of five samples with grain size based on the Wentworth Scale.	14
Figure 4. Ammonium (NH ₄ ⁺) and nitrite+nitrate (NO ₃ ⁻) concentrations (mean \pm SE) in the water column from the vegetated and non-vegetated site for each time interval. Mean of eight samples.	17
Figure 5. Ammonium (NH ₄ ⁺), nitrite+nitrate (NO ₃ ⁻) and particulate nitrogen concentrations (mean \pm SE) in the sediments from the vegetated and non-vegetated site for each time interval. Mean of eight samples.	19

LIST OF TABLES

	Page
Table 1. Initial concentrations of ammonium (NH_4^+), nitrite+nitrate (NO_3^-), and particulate nitrogen (PN) for the water column and sediments in Padilla Bay, Washington. Mean (\pm SE) of eight samples.	15
Table 2. Ammonium (NH_4^+), nitrite+nitrate (NO_3^-), and particulate nitrogen (PN) concentrations in the water column and sediments for untreated and NH_4^+ enriched cores from the vegetated and non-vegetated sites. Mean (\pm SE) of eight samples (untreated cores) and of five samples (enriched cores).	21

INTRODUCTION

In estuarine, coastal and oceanic environments, nitrogen is present in several different forms. Most of the nitrogen is in the form of N_2 , an essentially unreactive form, so the more biologically available forms (ammonium, nitrite, and nitrate) are in great demand, and are often limiting (Hecky and Kilham, 1988). However, ammonium and nitrate can be transported to estuarine and coastal environments via rivers and runoff from the surrounding land masses (Valiela, 1984). Uptake and regeneration of nitrogen in the water column and sediments also influence the availability of nitrogen species. In shallow systems, the exchange of dissolved nitrogen across the sediment-water interface is an important process affecting the chemical composition of the overlying water column (Callender and Hammond, 1982). In particular, nitrogen conversions in the sediment and in the overlying water column play a significant role in controlling water quality (Reddy *et al.*, 1988).

Benthic-pelagic coupling is an essential facet of the nitrogen cycle in shallow marine systems (Klump and Martens, 1983). Deposition of organic matter from the overlying water column enhances nitrogen transformations within the sediments. Nitrogen transformations in the sediments can result in a release of nitrogen to the overlying water column, or to nitrogen loss from the system (Valiela, 1984).

The flux of inorganic nitrogen across the sediment-water interface depends on the biological processes of ammonification, immobilization, nitrification, denitrification, and dissimilatory nitrate reduction to ammonium (Kaspar, 1983). These processes depend upon microorganisms that mediate the transformation of nitrogen through its many valence states (Atlas and Bartha, 1993; Kairesalo *et al.*, 1995). The microorganisms

present, and the nitrogen transformations undertaken by them are controlled by the physical and chemical conditions in the sediments and water column. Temperature, oxidation state, ammonium and nitrate availability, and the presence of hydrogen sulfide can act to inhibit or to enhance the nitrogen transformations. Temperature affects the rate of ammonification by affecting the rate of organic decay (Klump and Martens, 1987). The higher the temperature, the greater the rate of organic decay, and the more regeneration of ammonium (Valiela, 1984). The oxidation state of the sediments and water column also plays an important role in determining the nitrogen transformations that can occur. Ammonification and assimilatory nitrate reduction can occur under both aerobic and anaerobic conditions. Denitrification and dissimilatory nitrate reduction only occur under anaerobic conditions, while nitrification can only occur under aerobic conditions (Atlas and Bartha, 1993). In addition, *in situ* concentrations of ammonium and nitrate influence the processes which occur. Nitrification cannot proceed if ammonium is not present (Reddy *et al.*, 1989); likewise, denitrification cannot occur if nitrate is not present (Koike and Hattori, 1978). The presence of hydrogen sulfide in sediments inhibits nitrification (Joye and Hollibaugh, 1995; Sloth *et al.*, 1995), which can consequently repress denitrification (Seitzinger, 1988).

Many studies report that sediments are sinks and sites of nitrogen transformations (Propp *et al.*, 1980; Zeitzschel, 1980; Nixon, 1981; Valiela, 1983; DeLaune and Smith, 1987). In a study conducted by DeLaune *et al.* (1990) in a freshwater lake in Louisiana, the sediments served as buffer zones, removing large amounts of nitrogen and phosphorus from the water column. Valiela (1983) found that nitrogen in salt marsh sediments

constitutes up to 5% of the total weight, and that the sediments are the largest pool of nitrogen in the marsh. The sediments may also release nutrients, and may supply up to 100% of the nutrients required for primary production (Zeitschel, 1980). In the tidal Potomac river, Callender and Hammond (1982) found that benthic regeneration provided a significant fraction of the nutrients needed by primary producers during the summer months. Kaspar (1983) also suggests an export of inorganic nitrogen from the sediments to the overlying water column.

Seagrasses are known to influence the physical characteristics of the sediments by trapping and accumulating a high proportion of silt/clay particles and organic matter (Kenworthy *et al.*, 1982). The dense network of roots and rhizomes stabilizes the sediments and reduces erosion and the resuspension of bottom material (Orth, 1977), while the leaf canopy reduces the velocity and turbulence of the water (Fonseca *et al.*, 1982). These processes, in turn, may affect nitrogen cycling in the sediments (Caffrey and Kemp, 1990; Kenworthy *et al.*, 1982). Ammonification is enhanced by decomposition of the high amounts of trapped organic materials (Kemp *et al.*, 1984; Kenworthy and Thayer, 1984), while microbial activity is enhanced by the release of dissolved organic nitrogen from plant roots (Smith *et al.*, 1988). Nitrogen fixation and denitrification in seagrass beds may be enhanced by the release of labile dissolved organic compounds (Iizumi *et al.*, 1980; O'Neill and Capone, 1989). Denitrification may also be stimulated by the production of nitrate from nitrification (Koike and Sorensen, 1988). Nitrification may be stimulated by the release of oxygen from the eelgrass roots and rhizomes (Iizumi *et al.*, 1980).

The primary objective of this research was to measure and compare nitrogen exchange between the water column and sediments in vegetated and non-vegetated areas in Padilla Bay, Washington. Padilla Bay is a shallow embayment with a large expanse of seagrass beds and mudflats. It is subject to nutrient loading from the surrounding agricultural lands via runoff and the drainage sloughs that enter the bay, and has the potential for eutrophication (Bulthuis, 1993). Vegetated and non-vegetated sites in the bay were selected to test the hypothesis that nitrogen flux and transformations are different between the two sites. Specific questions included: 1) Are ammonium and nitrite+nitrate concentrations different between the water column and sediment porewaters? If so, then to what extent? 2) Are concentrations of ammonium and nitrite+nitrate different between vegetated and non-vegetated sites? 3) Is nitrogen being cycled differently at vegetated and non-vegetated sites? 4) Are the sediments acting as a sink or a source of nitrogen?

METHODS

Site description

Sediment cores were collected in Padilla Bay National Estuarine Research Reserve in Skagit County, Washington. Padilla Bay contains the largest contiguous seagrass meadow in Washington State with the dominant eelgrass species consisting of *Zostera marina* and *Zostera japonica* (Bulthuis, 1991). The distribution of eelgrass is not continuous; rather, it is patchy and intermixed with bare mudflats. The study sites were located in the intertidal zone on either side of Bayview Channel at 48° 29.077' N and 122° 29.829' W (Figure 1). The south side of the channel at this location is a bare mudflat, while the north side is a dense *Zostera marina* bed.

Site characterization

A 10 m x 20 m sampling site was established on each side of Bayview Channel by using permanent transects that were marked at 1-m intervals. At each site, *Z. marina* density was determined by counting turions in a 0.16 m² quadrat in 12 randomly chosen plots. Five sediment cores sampled to a depth of 10 cm were obtained from each of the two sites to determine the percent water, percent organic material, and grain size. The cores were homogenized at the laboratory, and a subsample of each was removed for the determination of percent water and percent organic material. The remaining core was stored at 5° C until grain size was determined. Percent organic material was determined by drying sediment samples to a constant weight at 105° C, and combusting the dried samples in a muffle furnace at 550° C for four hours. Percent water was determined by

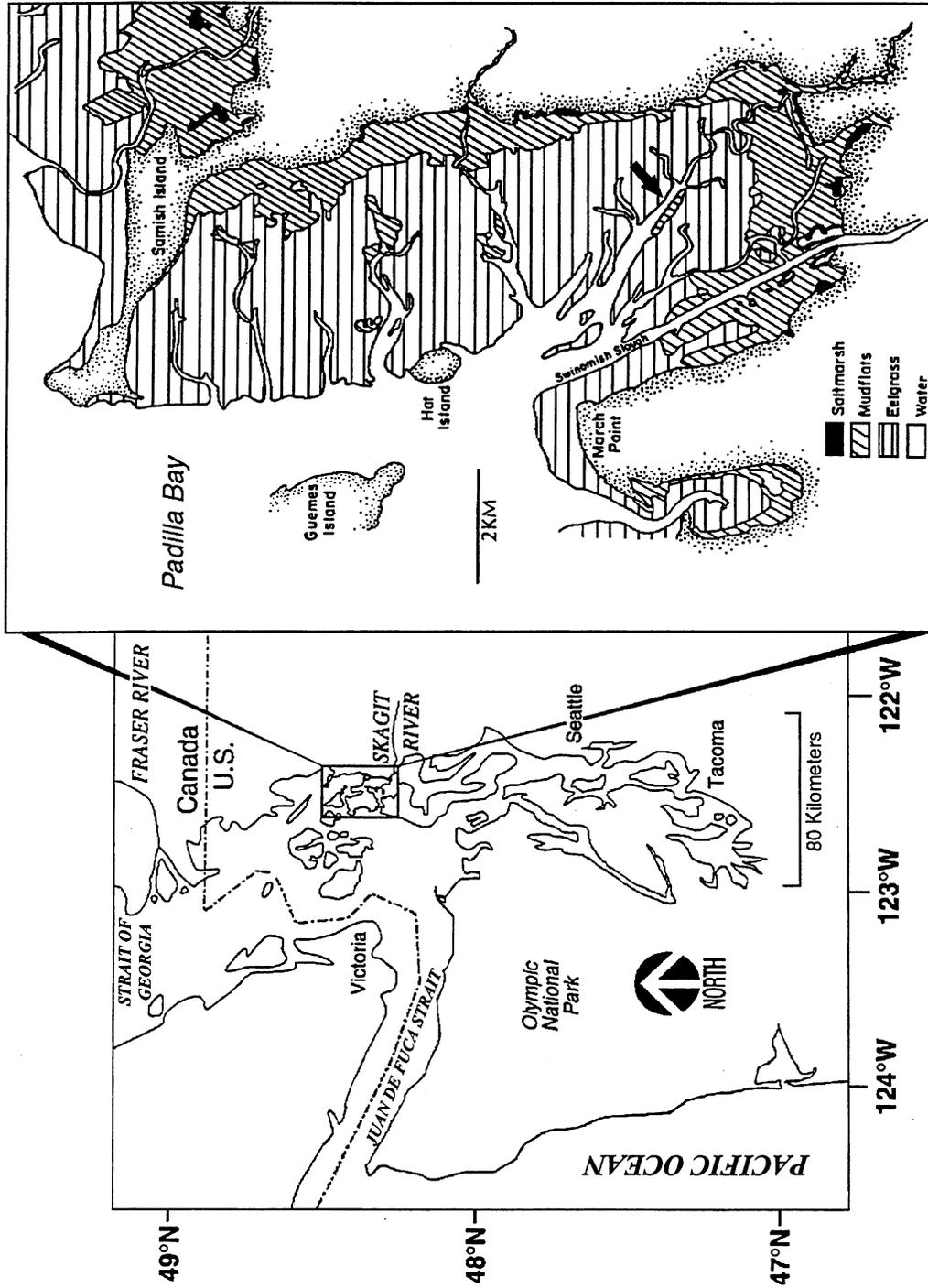


Figure 1. Map of Washington State showing location of Padilla Bay. The study site is indicated by an arrow on the right panel.

weighing the sediment sample before and after drying at 105° C. To determine the grain size distribution, the cores were wet sieved to separate the sand fraction (>62 µm) from the silt/clay fraction (<62 µm) and dried for 24 hours at 90° C. The dried sample was then shaken for 15 minutes through 1 mm, 500 µm, 250 µm, 125 µm and 63 µm sieves and the fractions weighed to the nearest 0.01 g (Puget Sound Estuary Program, 1990).

The slope, elevation difference and sediment temperature were also measured at each site. The slope of the sites was determined using a surveying instrument (transit) and stadia rod. The elevation difference between the two sites was determined by marking the sites simultaneously at low tide, surveying the height of each site at that low tide mark and taking the difference between the two.

Sampling procedures

Field

Ninety sediment cores were randomly collected within the 10 m x 20 m sampling sites on May 4, 1996. All cores were collected during the period of low tide (- 1.7 m). Half of the cores were obtained from the non-vegetated site, and half from the vegetated site. Sediments were sampled to a depth of 10 cm using 4.5-cm diameter hand-held buteryte core tubes. The cores were capped, taken to the laboratory and incubated in a continuously flowing, ambient seawater bath on a 16:8 hour, light:dark cycle.

Water column salinity, temperature, dissolved oxygen, and pH were determined in Bayview Channel using a Hydrolab. Irradiance was measured as photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$) using two LiCor quantum sensors: a cosine quantum sensor for above

water irradiance and a 4π quantum sensor for underwater irradiance. Ambient seawater was collected from the channel for addition to the cores in the laboratory.

Laboratory

Each core was filled with 500 ml of seawater collected at the site. Fifty cores (25 from the vegetated site and 25 from the non-vegetated site) received unfiltered ambient seawater, while the remaining 40 cores (20 from the vegetated site, 20 from the non-vegetated site) received filtered ($0.2\ \mu\text{m}$) ambient seawater. Incubation of all cores began within five hours of collection. The cores were incubated in a continuously flowing, ambient ($13^\circ\ \text{C}$) seawater bath on a 16:8 hour, light:dark cycle.

Of the 50 cores with unfiltered ambient seawater, 10 (5 vegetated, 5 non-vegetated) were enriched with $10\ \mu\text{M}\ \text{NH}_4\text{Cl}$ to double the water column concentration of NH_4^+ . The cores were enriched to see if a surplus of NH_4^+ would change the direction or the rate of the exchange of nitrogen.

Sampling was conducted at time intervals of 0, 6, 12, 24, and 48 hours. At each interval, 4 replicate cores of each treatment (vegetated unfiltered, vegetated filtered, non-vegetated unfiltered, and non-vegetated filtered) were removed from the incubation tank. The water column in each core was siphoned and 20 ml were filtered through a Whatman GF/F filter ($0.7\ \mu\text{m}$ nominal pore size). The filtrate was then frozen at $-20^\circ\ \text{C}$ for determination of NH_4^+ and $\text{NO}_2^- + \text{NO}_3^-$ concentrations. The sediment from each core was also frozen at $-20^\circ\ \text{C}$ for determination of NH_4^+ , $\text{NO}_2^- + \text{NO}_3^-$, and particulate nitrogen. The NH_4^+ enriched cores were removed at the 24-h sampling interval and

treated as described above. All cores were stirred with a glass rod at each time interval to diminish the formation of any nutricline within the water column. Dissolved oxygen levels were monitored with a YSI model 57 oxygen meter at each sampling interval during the incubation in a subset of 10 cores (5 vegetated and 5 non-vegetated).

Interstitial porewaters were extracted from the sediments by centrifuging for 30 minutes at 15000 rpm using a Sorval Model RC 26⁺ high-speed centrifuge. The porewater was removed, filtered through a Whatman GF/F filter (0.7 μm nominal pore size), and immediately frozen at -20°C for the determination of NH_4^+ and $\text{NO}_2^- + \text{NO}_3^-$. The sediments were then re-frozen until the determination of particulate nitrogen was conducted.

Chemical analysis of water, porewater and sediments

All water column and interstitial porewater NH_4^+ concentrations were determined using the alternative phenolhypochlorite method for NH_4^+ (Parsons *et al.*, 1984), while $\text{NO}_2^- + \text{NO}_3^-$ concentrations were determined using an Alpkem Autoanalyzer. The following range of standards was used: water column NH_4^+ (0.75- 48 $\mu\text{M-N}$), $\text{NO}_2^- + \text{NO}_3^-$ (0-20 $\mu\text{M-N}$); porewater NH_4^+ (48 - 120 $\mu\text{M-N}$), $\text{NO}_2^- + \text{NO}_3^-$ (0-35 $\mu\text{M-N}$). All nutrient concentrations are reported as $\text{NH}_4^+ \text{-N}$ and $\text{NO}_2^- + \text{NO}_3^- \text{-N}$, and are within the detection limits of the methods used.

Particulate nitrogen in the sediments was determined using a Lehman Labs, Inc. CE 440 Elemental Analyzer. The sediments were dried at 90°C to a constant weight and homogenized with a mortar and pestle. Samples were weighed on a CAHN electrobalance

model 4700, and approximately 5 mg of sample were placed into silver weigh boats. Samples were then fumed with HCl for 24 hours to remove all inorganic carbon, after which they were placed in a drying oven at 90°C for 24 hours to drive off any remaining HCl. Samples were placed in the elemental analyzer and combusted at 980° C, then reduced at 700° C. Particulate nitrogen concentrations were then calculated from a standard curve based on acetanalide.

Experimental design and data analysis

The experiment was a 2x2x2x5 factorial design with the following factors: 2 sites (vegetated and non-vegetated), 2 treatments (unfiltered and filtered water columns), 2 origins (water column and porewaters), and 5 time intervals (0, 6, 12, 24 and 48 hrs). All factors were fixed and represented in the linear model:

$$Y_{ijklm} = \mu + S_i + F_j + SF_{ij} + O_k + SO_{ik} + FO_{jk} + SFO_{ijk} + T_l + ST_{il} + FT_{jl} + OT_{kl} + SFOT_{ijkl} + e_{(ijkl)m}$$

where

- S = site (i = 1..2, fixed)
- F = treatment (j = 1..2, fixed)
- O = origin (k= 1..2, fixed)
- T = time (l = 1..5, fixed)
- 4 replicates (m = 1..4, random).

When the data were analyzed, the filtered and unfiltered cores were not significantly different from one another, so the data were pooled, and that factor was removed from the model (Appendix A and B for ANOVA tables). The data were then analyzed by the

following linear model:

$$Y_{ijkl} = \mu + S_i + O_j + SO_{ij} + T_k + ST_{ik} + OT_{jk} + SOT_{ijk} + e_{(ijkl)}$$

where

- S = site (i = 1..2, fixed)
- O = origin (j = 1..2, fixed)
- T = time (k = 1..5, fixed)
- 4 replicates (l = 1..4, random).

The data were analyzed by ANOVA using the statistical software Statistix. General contrasts were used to examine significant interactions and simple main effects. The error rate was controlled for non-orthogonal contrasts by using a Bonferroni sequential correction. All other significance levels were set at 0.05.

RESULTS

Zostera marina density at the vegetated site was uniform with an average of 109 turions/m² (n quadrats = 12; SE±0.96), while the non-vegetated site was completely devoid of eelgrass. Macroalgae were not abundant at either of the sites on the sampling date; however, benthic microalgae were present. The vegetated site had a significantly higher amount of organic matter (p=0.0000) and a significantly higher water content (p=0.0000) than the non-vegetated site (Figure 2). There was also a positive correlation between percent organic matter and percent water at each of the two sites (r=0.959). The non-vegetated site was 1 m higher elevation than the vegetated site. At both sites, the predominant grain sizes were fine and very fine sand. However, the vegetated site had a higher proportion of silts and clays (Figure 3). The higher proportion of silts and clays was positively correlated with a higher water content (r=0.859), and a higher amount of organic material (r=0.839).

The water column in the channel was isothermal (12.9° C). At the surface, there was a freshwater lens (5 ppt) that was well oxygenated (11.3 mg l⁻¹). The rest of the water column was isohaline (26.4 ppt), and well oxygenated (10.2 mg l⁻¹).

Initial water column ammonium concentrations were not significantly different in cores from the vegetated and non-vegetated sites, nor were nitrite+nitrate concentrations (Table 1). Initial porewater concentrations of ammonium and nitrite+nitrate were not significantly different between sites. Within the cores from each site, porewater concentrations of ammonium were 25 to 50 times greater than water column values, while porewater nitrite+nitrate concentrations were twice as high. Both ammonium and

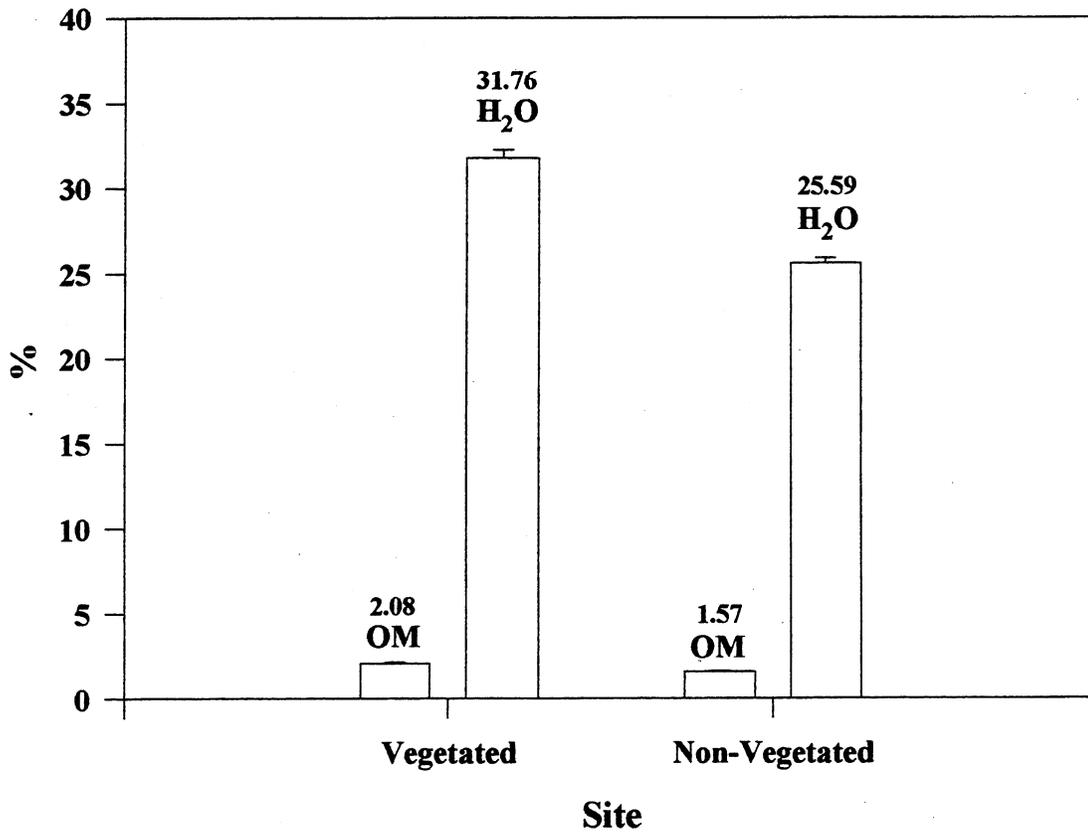


Figure 2. Percent organic matter (OM) and percent water (H₂O) in the sediments from the vegetated and non-vegetated sites in Padilla Bay, Washington. Mean (\pm SE) of five samples.

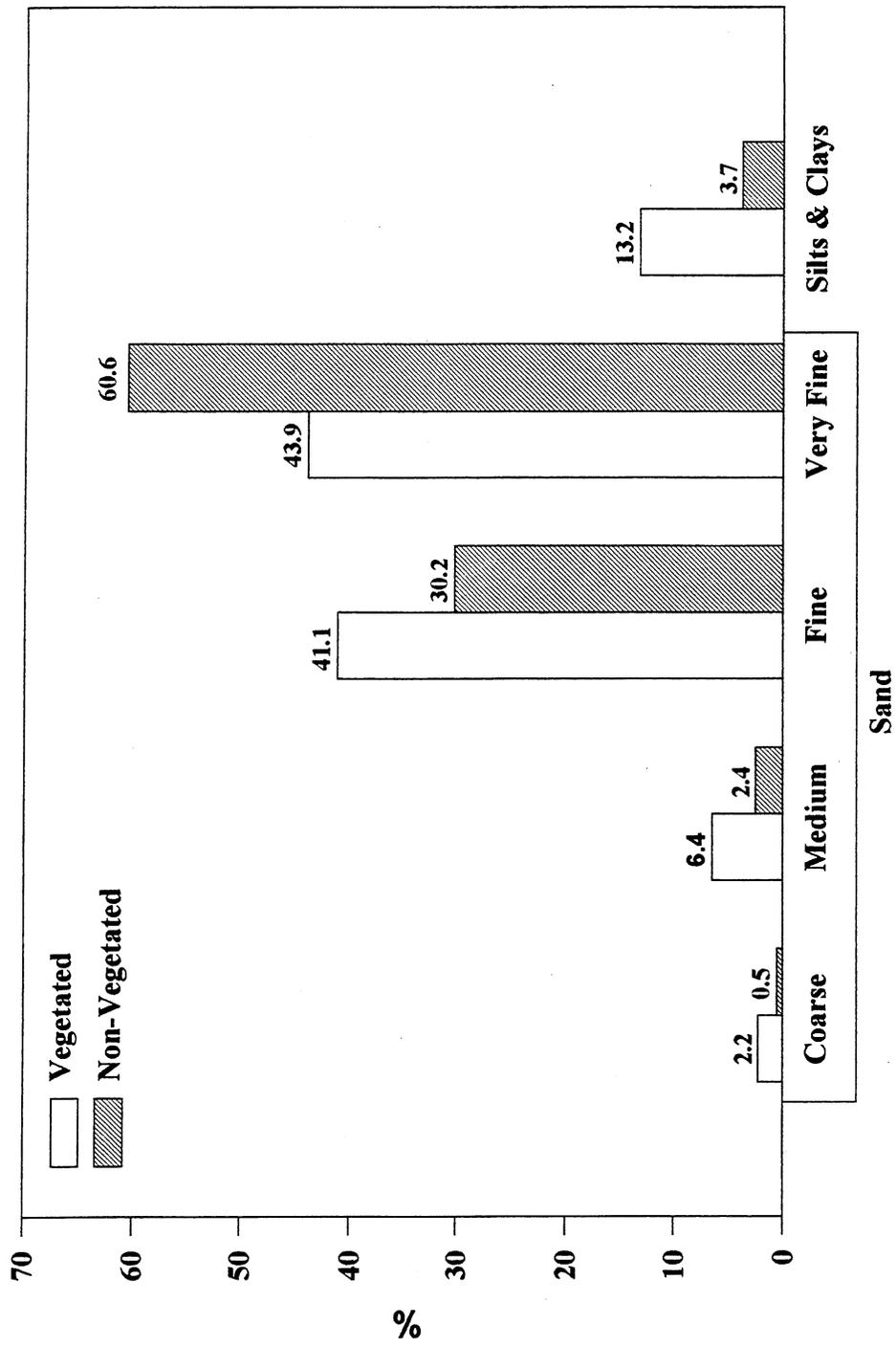


Figure 3. Grain size distribution at the vegetated and non-vegetated sites. Mean of five samples with grain size based on the Wentworth Scale.

Table 1. Initial concentrations of ammonium (NH₄⁺), nitrite+nitrate (NO₃⁻), and particulate nitrogen (PN) for the water column and sediments in Padilla Bay, Washington. Mean (±SE) of eight samples.

	Water Column		Sediments	
	Vegetated	Non-Vegetated	Vegetated	Non-Vegetated
NH ₄ ⁺ (μM)	2.03 (±0.90)	1.07 (±0.25)	55.38 (±3.94)	59.56 (±3.47)
NO ₃ ⁻ (μM)	4.67 (±0.33)	4.42 (±0.32)	9.04 (±1.54)	10.17 (±1.51)
PN (μM)	--	--	72000 (±2001.0)	50460 (±3578.8)

nitrite+nitrate concentrations were significantly higher in the porewaters than in the water column. Particulate nitrogen concentrations in the sediments were significantly greater in the vegetated sediments than in the non-vegetated sediments (Table 1). Overall, dissolved inorganic nitrogen ($\text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^-$) constituted less than 1% of the total measured nitrogen, while particulate nitrogen contributed greater than 99% of the total measured nitrogen.

Laboratory incubation results

Water column

There was no significant change in NH_4^+ or $\text{NO}_2^- + \text{NO}_3^-$ concentrations over time in the cores from the two sites, nor was there a significant difference in concentrations between the two sites (Figure 4). There was however, an overall trend of higher NH_4^+ levels and lower $\text{NO}_2^- + \text{NO}_3^-$ levels at the end of the incubation than at the beginning in cores from both sites.

Sediments and porewaters

In the porewaters of the cores from the vegetated site, there was a significant increase in NH_4^+ that began at 24 hours ($p=0.0020$) and continued through 48 hours ($p=0.0000$). In the cores from the non-vegetated site, there was no significant difference between initial (time 0) and final (48 hours) concentrations of NH_4^+ . The NH_4^+ concentrations did fluctuate during the incubation with a significant decrease ($p=0.0007$) at the 6-h sampling interval. NH_4^+ concentrations then increased at the 12-h and

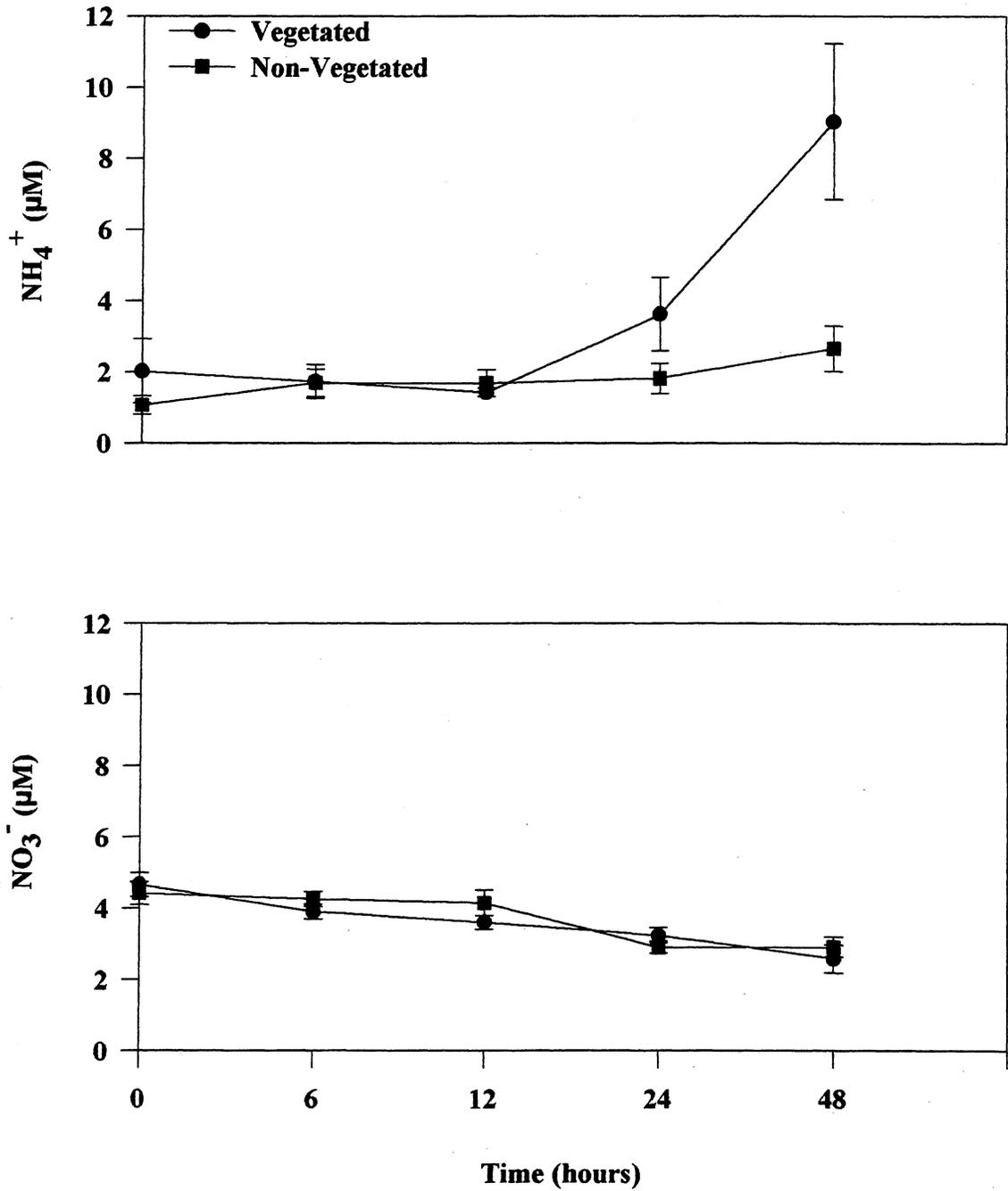


Figure 4. Ammonium (NH_4^+) and nitrite+nitrate (NO_3^-) concentrations (mean \pm SE) in the water column from the vegetated and non-vegetated site for each time interval. Mean of eight samples.

24-h intervals, and were significantly higher by the 48-h interval than at the 6-h interval. NH_4^+ concentrations were similar in the porewaters of cores from the two sites except at the 48-h interval when the concentrations in the vegetated cores were significantly higher than in the non-vegetated cores ($p=0.0214$) (Figure 5).

In the porewaters of the cores from the vegetated site, there was no significant difference between initial (time 0) and final (48 hours) $\text{NO}_2^- + \text{NO}_3^-$ concentrations. There were significant changes during the incubation with a significant increase ($p=0.0000$) in $\text{NO}_2^- + \text{NO}_3^-$ at 12 hours, followed by a significant decrease from the 12-h value ($p=0.0004$) at 24 hours. At 48 hours, $\text{NO}_2^- + \text{NO}_3^-$ concentrations increased, but were not significantly different from 24 hours (Figure 5). In cores from the non-vegetated site, $\text{NO}_2^- + \text{NO}_3^-$ concentrations were significantly higher at the 48-h interval than the means of all other time intervals ($p=0.0000$). There was also a significant increase in $\text{NO}_2^- + \text{NO}_3^-$ concentrations between the 0 and 6-h sampling intervals ($p=0.0007$), followed by a significant decrease at the 12 and 24-h intervals ($p=0.0006$ and $p=0.0009$, respectively).

The concentration of $\text{NO}_2^- + \text{NO}_3^-$ in the porewaters was not significantly different between sites except at 6 and 12 hours ($p=0.0046$ and $p=0.0000$, respectively). This significant difference contributed to a significant 3-way interaction between site, time and sample (Appendix B2).

Particulate nitrogen was significantly higher ($p=0.0000$) in cores from the vegetated site than from the non-vegetated site for all sampling intervals except at the 24-h time interval (Figure 5). Within cores from each site, particulate nitrogen levels

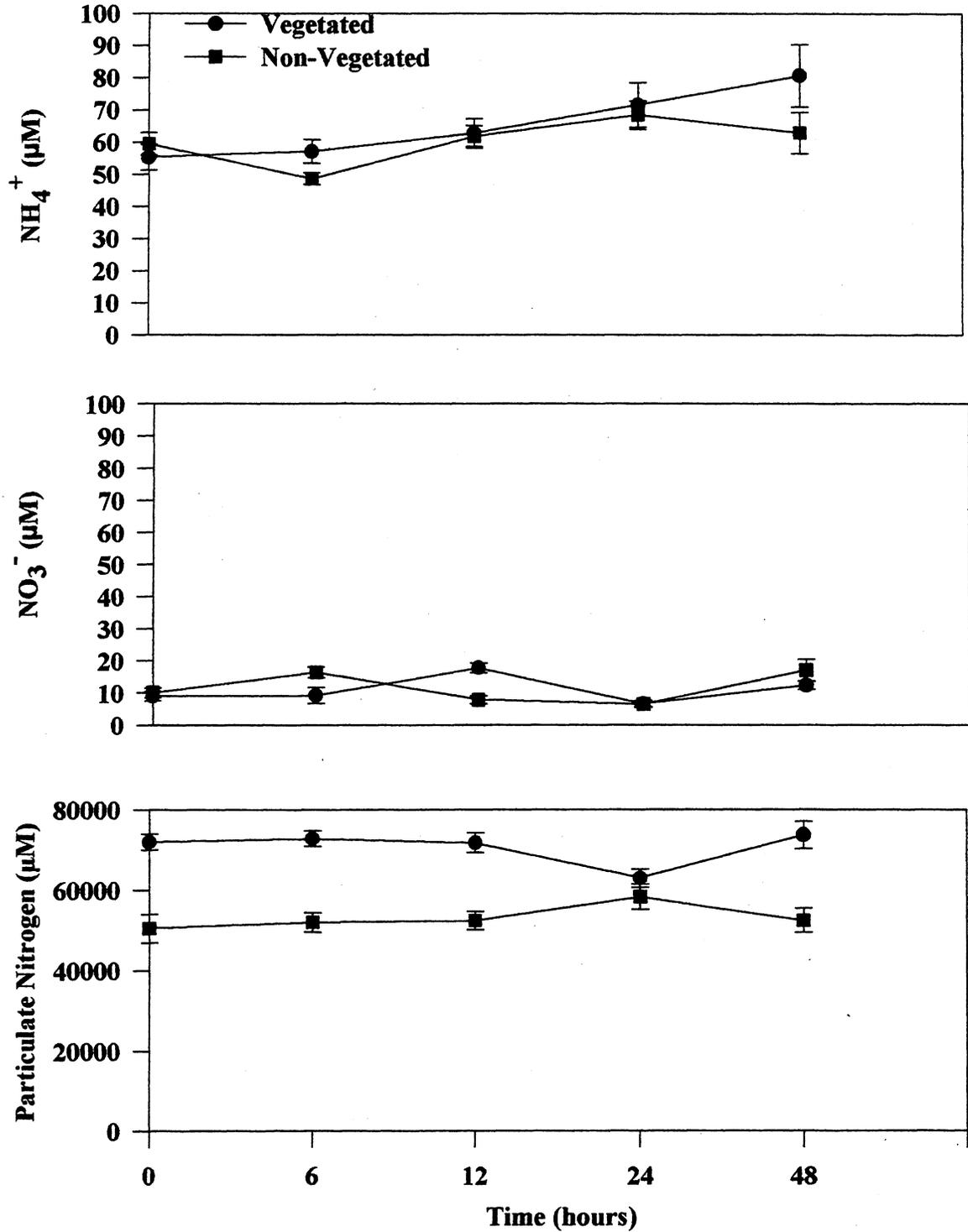


Figure 5. Ammonium (NH_4^+), nitrite+nitrate (NO_3^-) and particulate nitrogen concentrations (mean \pm SE) in the sediments from the vegetated and non-vegetated site for each time interval. Mean of eight samples.

remained essentially unchanged, but dissolved inorganic nitrogen levels increased during the incubation.

Effect of NH_4^+ addition on NH_4^+ , $\text{NO}_2^- + \text{NO}_3^-$, and particulate nitrogen concentrations

The enrichment had no significant effect on NH_4^+ concentrations in the water column or porewaters of either site. There was also no significant effect on $\text{NO}_2^- + \text{NO}_3^-$ concentrations in the water column of either site. In the porewaters, there was a significantly higher amount of $\text{NO}_2^- + \text{NO}_3^-$ in the enriched non-vegetated cores ($p=0.0000$) than in all other cores. $\text{NO}_2^- + \text{NO}_3^-$ porewater concentrations were also significantly higher than water column concentrations in the non-vegetated enriched core ($p=0.0000$), but were not significantly different in the other treatments. Particulate nitrogen concentrations were significantly greater in the enriched cores than in the untreated cores at the vegetated site ($p=0.0451$), but were significantly less at the non-vegetated site ($p=0.0017$). The vegetated enriched cores had a significantly greater amount of nitrogen than the non-vegetated enriched cores ($p=0.0000$) (Table 2).

Table 2. Ammonium (NH₄⁺), nitrite+nitrate (NO₃⁻), and particulate nitrogen (PN) concentrations in the water column and sediments for untreated and NH₄⁺ enriched cores from the vegetated and non-vegetated sites. Mean (±SE) of eight samples (untreated cores) and of five samples (enriched cores).

	Water Column		Sediments	
	Vegetated	Non-Vegetated	Vegetated	Non-Vegetated
NH₄⁺ (μM)				
Enriched	3.62 (±1.03) 11.33 (±2.60)	1.82 (±0.23) 9.11 (±1.34)	71.62 (±6.86) 73.12 (±3.43)	68.51 (±4.42) 72.74 (±11.18)
NO₃⁻ (μM)				
Enriched	3.24 (±0.22) 3.34 (±0.21)	2.91 (±0.18) 2.94 (±0.24)	6.60 (±0.82) 4.59 (±0.24)	6.46 (±1.08) 13.60 (±2.85)
PN (μM)	-	-	62970 (±2269.6)	58320 (±3169.9)
Enriched	-	-	68260 (±2079.2)	45830 (±3269.8)

DISCUSSION

Zostera marina distribution, turion densities, and sediments consisting of greater than 70% sand were consistent with reports from earlier studies in Padilla Bay (Bulthuis, 1991; Turner, 1980; Webber *et al.*, 1987). The vegetated site contained a larger quantity of the silt/clay size fraction, and a significantly greater fraction of organic matter than did the non-vegetated site. The higher amount of silts and clays as well as organic material at the vegetated site is characteristic of sediments in habitats containing seagrasses such as *Z. marina*. The seagrasses alter the physical environment by stabilizing the sediments and reducing erosion. They also slow water velocities and minimize turbulence leading to greater deposition of fine-grained particles. Quieter waters also lead to enhanced retention of organic matter from the plants' leaf deposition and root and rhizome biomass (Fonseca *et al.*, 1982; Kenworthy *et al.*, 1982; Orth 1977).

Initial water column ammonium and nitrite+nitrate concentrations were similar to those reported in studies by Bernhard (1993) and Williams and Ruckelshaus (1993), but were lower than those reported by Cassidy and McKeen (1986). Initial porewater concentrations of ammonium averaged 57 μM , which is lower than the 100 μM reported by Williams and Ruckelshaus (1993). They reported average concentrations from cores taken in April and August, and the means may mask seasonal and interannual changes in concentrations. In general, porewater concentrations of both ammonium and nitrite+nitrate were within the range of concentrations reported for sediments in other eelgrass beds (Hemminga *et al.*, 1994; Iizumi *et al.*, 1980; Kenworthy *et al.*, 1982).

Particulate nitrogen concentrations in the sediments showed the same trend as seen in percent organic material. The vegetated site had a significantly greater concentration of particulate nitrogen than did the non-vegetated site. Kenworthy *et al.* (1982) determined that the percent organic matter of coastal sediments was positively correlated to the percent total nitrogen, of which particulate nitrogen was probably the largest pool. Particulate nitrogen was the largest pool of nitrogen in this study, and has been shown to be the largest pool of nitrogen in many systems (DeLaune *et al.*, 1990; Devol and Christensen, 1993; Valiela, 1983).

Nitrogen transformations and cycling

Water column

Although ammonium and nitrite+nitrate concentrations did not vary significantly over the 48-h incubation period, ammonium increased and nitrite+nitrate decreased in the water column of cores from both sites. The decrease in nitrite+nitrate concentrations may reflect nitrate uptake by phytoplankton and benthic microalgae. Henriksen *et al.* (1980) demonstrated that benthic algae take up nitrate in the presence of ammonium. However, ammonium is the preferred form of nitrogen for uptake by phytoplankton and bacteria (Valiela, 1984). The apparent discrepancy may be explained by microbial regeneration of nitrogen and population dynamics. Zooplankton excrete nitrogen as ammonium, and bacteria break down organic nitrogen to ammonium through ammonification (Lalli and Parsons, 1993; Valiela, 1984). At the same time, grazing by zooplankton can control phytoplankton populations, allowing the zooplankton population to increase (Sherr and

Sherr, 1994). The result is greater rates of ammonium excretion than ammonium uptake by plankton in the water column. Another possible explanation for the decrease in nitrite+nitrate concentrations and the increase in ammonium levels may be denitrification and dissimilatory nitrate reduction. However, both of these processes require hypoxic or anoxic conditions. Denitrification occurs only when oxygen levels reach 0.2 mg/l or less (Seitzinger, 1988). The oxygen levels in the water column above the cores did not drop below 8 mg l⁻¹ during the 48-h incubation, and the oxidized layer did not disappear from the sediments, so this is an unlikely explanation. Seitzinger (1988) also indicates that nitrate in an aerobic water column is not a major source for denitrification; rather most of the nitrate is supplied by the sediments.

In addition to the regeneration of ammonium by heterotrophs in the water column, ammonium was probably being released to the water column from the sediments. Under calm conditions, concentration gradients between the sediment-water interface drive the flux of dissolved nutrients (Reddy *et al.*, 1996). Ammonium concentrations in the porewaters from both sites were 25 to 50x higher than in the water column, creating a large concentration gradient. However, there was no significant increase in water column ammonium concentrations over the incubation. The presence of benthic microalgae, the length of the incubation, the lack of water circulation in the cores or some combination of these factors may account for the discrepancy. Uptake of ammonium by benthic microalgae removes a significant proportion of the nitrogen flux across the sediment-water interface (Sundbäck and Granéli, 1988; Sundbäck *et al.*, 1991). The length of the incubation period coupled with the lack of regular water circulation could have also

resulted in a decrease in the concentration gradients (Miller-Way and Twilley, 1996). However, there was no sign of a decline in the concentration gradient as final porewater values were higher than initial concentrations in vegetated cores, and they were unchanged in non-vegetated cores. Continuous stirring of the cores would have maintained the concentration gradients, but the effect of stirring on nutrient flux is not well known. Boynton *et al.* (1981) found that stirring enhanced sediment exchange rates, while Callender and Hammond (1982) determined that there was no difference in oxygen or nutrient fluxes in stirred and unstirred chambers. In addition, stirring may create deleterious effects such as changing the thickness of the oxic zone in the sediments (Höhener and Gächter, 1994) or by resuspending sediments, causing a release of nutrients (Reddy *et al.*, 1996).

Sediments and porewaters

The amount of organic material in the sediments influences oxygen consumption, rates of ammonification, and the efflux of ammonium from the sediments (Kelly and Nixon, 1984; Short, 1983; Sloth *et al.*, 1995). The cores from the vegetated site contained a significantly greater amount of organic material than did the cores from the non-vegetated site. While no redox measurements were taken, the sediments in the cores from the vegetated site were black and smelled of hydrogen sulfide, indicating reduced or anoxic conditions. This is typical of the sediments in eelgrass beds (Williams and Ruckelshaus, 1993) which often contain more organic material, leading to higher rates of oxygen consumption. In the cores from the non-vegetated site, the sediments were brown

and had no noticeable odor of hydrogen sulfide, indicating more oxygenated conditions than in the vegetated cores. The lower amount of organic material in the non-vegetated sediments may have led to a lower oxygen demand. Benthic microalgae in the non-vegetated sediments may have influenced the oxidation state of the sediments. Although the density and composition of the benthic microalgae were not determined, the non-vegetated site appeared to support a greater number of diatoms. Oxygen produced by the benthic microalgae community can diffuse downward into the sediments, creating a thick layer of oxygenated sediments (Risgaard-Petersen *et al.*, 1994). The oxidation state of the sediments plays an important role in determining the nitrogen transformations that can occur. Ammonification and assimilatory nitrate reduction can occur under both aerobic and anaerobic conditions. Denitrification and dissimilatory nitrate reduction only occur under anaerobic conditions, while nitrification can only occur under aerobic conditions (Atlas and Bartha, 1993).

At the end of the incubation, ammonium concentrations in the porewaters of the cores from the vegetated site were significantly higher than initial porewater concentrations, while nitrite+nitrate levels were significantly higher in the cores from the non-vegetated site. These results indicate that the dominant nitrogen transformations taking place in the cores from vegetated and non-vegetated sites were different. The difference between sites is directly related to the amount of organic material present and to the oxidation state of the sediments.

Ammonification was the dominant nitrogen transformation occurring in the sediment cores from the vegetated site. However, Caffrey and Kemp (1990) found that

rates of ammonification, nitrification, and denitrification exhibit seasonality, so it is inappropriate to imply that ammonification will always be the dominant process in the eelgrass beds of Padilla Bay. Many studies have shown, however, that ammonification rates are consistently higher in vegetated sediments than in bare sediments. Boone *et al.* (1986) report higher ammonification rates in sediments vegetated with *Zostera capricorni* than in non-vegetated sediments. Caffrey and Kemp (1990) also report significantly higher rates of ammonification in vegetated (*Z. marina*) sediments than in adjacent bare sediments. In their study, ammonification rates surpassed plant demand for ammonium, and ammonium accumulated in the vegetated sediments. Ammonium also accumulated in the vegetated sediments of this study, indicating extensive degradation of organic nitrogen.

Nitrification appears to be the dominant process in the sediment cores from the non-vegetated site. Nitrate levels in the porewaters increased significantly during the 48-h incubation period, while ammonium levels remained unchanged or declined. This indicates oxidation of ammonium to nitrate via nitrification. These data must be interpreted carefully because Caffrey and Kemp (1990) report that nitrification potentials are not related to ammonium concentrations or ammonification rates. However, nitrifying bacteria thrive in well oxygenated, sandy sediments with high ambient concentrations of ammonium (Koike and Hattori, 1978). Kaspar (1983) reports a greater accumulation of nitrate in sandy sediments than in sediments with a higher silt/clay content. The highest rates of nitrification have been reported in sediments with low to moderate levels of organic material (Billen, 1982; Sloth *et al.*, 1995).

The sediments are a source of nitrogen in cores from both the vegetated and non-vegetated sites during the spring. However, the role of the sediments and the relative importance of specific nitrogen transformations in the sediments may depend on the environmental conditions. The oxidation of the sediments and the availability of particular nitrogen species will vary with the seasons, organic loading and distribution, growth stage of the macrophytes, abundance of benthic algae, and oxygen diffusion.

Benthic-pelagic coupling

A large concentration gradient for both ammonium and nitrite+nitrate existed between the sediment porewaters and the overlying water column. The large difference in concentrations should have resulted in the diffusion of ammonium and nitrite+nitrate across the sediment-water interface. However, there was very little exchange between the sediments and water column. The apparent lack of exchange may be attributed to several factors. The incubation may need to be longer than 48 hours to detect changes. Overnell *et al.* (1995) determined that the nutrient fluxes between the sediments and water column were small, and that incubation needed to proceed for several days before a measurable change in nutrient concentrations occurred. In this study, ammonium levels were noticeably higher in the water column of cores from the vegetated site by the 48-h sampling interval. At the same time, there was also a significant accumulation of ammonium in the sediment porewaters. The increase at 48 hours may have been the beginning of the measurable flux of nitrogen from the sediments. Another possible explanation for the apparent lack of exchange is that the nitrogen flux from the porewaters

was diluted by the overlying water column. The volume of the water column in the incubated cores was much greater than that of the porewaters, and so may have masked any flux from the sediments. As discussed earlier, uptake by benthic microalgae and the breakdown of concentration gradients may have also masked or slowed the flux across the sediment-water interface.

Under more natural conditions, nitrogen exchange between the sediments and water column is facilitated by the hydraulic action of the tides which flush the interstitial waters (Harvey *et al.*, 1995; Malan and McLachlan, 1991). Sediment topography also influences the rate of nutrient exchange across the sediment-water interface.

Topographical features such as biogenic mounds and tracks, and geological features such as ripples affect boundary layer flow (Vogel, 1983). The interaction of the boundary layer and topography can create pressure differences that lead to upwelling of porewaters from the sediments to the surface (Ziebis *et al.*, 1996). Bioturbation by macrofauna can also enhance nitrogen flux from the sediments by resuspending sediments, and oxygenating sediments, enhancing nitrification rates (Caffrey, 1995; Yamada and Kayama, 1987). McCaffrey *et al.* (1980) estimated that bioturbation releases as many nutrients as, and perhaps more than, diffusion.

Conclusion

Although there was no measurable nitrogen exchange across the sediment-water interface, sediment porewater concentrations of ammonium and nitrite+nitrate were much greater than water column values. In cores from both the vegetated and non-vegetated

sites, the sediments are acting as a source of nitrogen to the porewaters. However, the transformations taking place in cores from the two sites are different. In the vegetated cores, ammonification is the dominant process, while nitrification is the dominant process in the non-vegetated cores. However, the role of the sediments and the transformations taking place within them may change depending on the season, organic loading, oxidation state of the sediments, the growth stage of the macrophytes, and the abundance of benthic algae.

LITERATURE CITED

- Atlas, R. M. and R. Bartha. 1993. Microbial Ecology. 3rd edition. Benjamin/Cummings Publishing Co., Inc, Redwood City, CA.
- Bernhard, A. E. 1993. Nutrient limitation of phytoplankton in Padilla Bay. M.S. Thesis. Western Washington University. 73 pp. Bellingham, Washington. Padilla Bay Estuarine Research Reserve Reprint Series No. 19 (Reprinted 1994).
- Billen, G. 1982. An idealized model of nitrogen recycling in marine sediments. Am. Jour. Sci. 282: 512-541.
- Boone, P. I., D. J. W. Moriarty and P. G. Saffigna. 1986. Rates of ammonium turnover and the role of amino-acid deamination in seagrass (*Zostera capricorni*) beds of Moreton Bay, Australia. Mar. Biol. 91: 259-268.
- Boynton, W. R., W. M. Kemp, C. G. Osborne, K. R. Kaumeyer, and M. C. Jenkins. 1981. Influence of water circulation rate on in situ measurements of benthic community respiration. Mar Biol 65: 185-190.
- Bulthuis, D. A. 1991. Distribution of habitats and summer standing crop of seagrasses and macroalgae in Padilla Bay, Washington, 1989. Washington State Department of Ecology, Padilla Bay National Estuarine Research Reserve Technical Report No. 2., Mount Vernon, Washington, 35 pp.
- Bulthuis, D. A. 1993. Review of water quality data in the Padilla Bay/Bay View watershed. Washington State Department of Ecology, Padilla Bay National Estuarine Research Reserve Technical Report No. 10, Mount Vernon, Washington. 72 pp.
- Caffrey, J. M. 1995. Spatial and seasonal patterns in sediment nitrogen remineralization and ammonium concentrations in San Francisco Bay, California. Estuaries 18: 219-233.
- Caffrey, J. M. and W. M. Kemp. 1990. Nitrogen cycling in sediments with estuarine populations of *Potamogeton perfoliatus* and *Zostera marina*. Mar. Ecol. Prog. Ser. 66: 147-160.
- Callender, E. and D. E. Hammond. 1982. Nutrient exchange across the sediment-water interface in the Potomac River estuary. Estuar. Coast. Shelf Sci. 15: 395-413.

- Cassidy, P. M. and G. L. McKeen. 1986. Padilla Bay baseline water quality record. Report to NOAA/OCRM/MEMD by Western Wash. Univ., Shannon Point Marine Center. 472 pp. Anacortes, Washington. Padilla Bay National Estuarine Research Reserve Reprint Series No. 2, 1990.
- DeLaune, R. D., C. W. Lindau, R. S. Knox and C. J. Smith. 1990. Fate of nitrogen and phosphorus entering a gulf coast freshwater lake: A case study. *Water Res. Bull.* 26: 621-631.
- DeLaune, R. D. and C. J. Smith. 1987. Simultaneous determination of nitrification and nitrate reduction in sediment-water columns by nitrate-15 dilution. *J. Environ. Qual.* 16: 227-230.
- Devol, A. H. and J. P. Christensen. 1993. Benthic fluxes and nitrogen cycling in sediments of the continental margin of the eastern North Pacific. *J. Mar. Res.* 51: 345-372.
- Fonseca, M. S., J. S. Fisher, J. C. Zieman and G. W. Thayer. 1982. Influence of the seagrass, *Zostera marina* L., on current flow. *Estuar. Coast. Shelf Sci.* 15: 351-364.
- Harvey, J. W., R. M. Chambers and J. R. Hoelscher. 1995. Preferential flow and segregation of porewater solutes in wetland sediment. *Estuaries* 18: 568-578.
- Hecky, R. E. and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Limnol. Oceanogr.* 33: 796-822.
- Hemminga, M. A., B. P. Koutstaal, J. van Soelen and A. A. A. Merks. 1994. The nitrogen supply to intertidal eelgrass (*Zostera marina*). *Mar. Biol.* 118: 223-227.
- Henriksen, K., J. Hanson and T. H. Blackburn. 1980. The influence of benthic infauna on exchange rates of inorganic nitrogen between sediment and water. *Ophelia* 1: 249-256.
- Höhener, P. and R. Gächter. 1994. Nitrogen cycling across the sediment-water interface in an eutrophic, artificially oxygenated lake. *Aquat. Sci.* 56: 115-132.
- Iizumi, H., A. Hattori and C. P. McRoy. 1980. Nitrate and nitrite in interstitial waters of eelgrass beds in relation to the rhizosphere. *J. exp. mar. Biol. Ecol.* 47: 191-201.
- Joye, S. B. and J. T. Hollibaugh. 1995. Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. *Science* 270: 623-625.

- Kairesalo, T., L. Tuominen, H. Hartikainen and K. Rankinen. 1995. The role of bacteria in the nutrient exchange between sediment and water in a flow-through system. *Microb. Ecol.* 29: 129-144.
- Kaspar, H. F. 1983. Denitrification, nitrate reduction to ammonium, and inorganic nitrogen pools in intertidal sediments. *Mar. Biol.* 74: 133-139.
- Kelly, J. R. and S. W. Nixon. 1984. Experimental studies of the effect of organic deposition on the metabolism of a coastal marine bottom community. *Mar. Ecol. Prog. Ser.* 17: 157-169.
- Kemp, W. M., W. R. Boynton, R. R. Trilley, J. C. Stevenson and L. G. Ward. 1984. Influences of submerged vascular plants on ecological processes in upper Chesapeake Bay. In V. S. Kennedy (ed.), *The estuary as a filter*. Academic Press, New York.
- Kenworthy, W. J. and G. W. Thayer. 1984. Production and decomposition of the roots and rhizomes of seagrasses, *Zostera marina* and *Thalassia testudinum*, in temperate and subtropical marine ecosystems. *Bull. mar. Sci.* 35: 364-379.
- Kenworthy, W. J., J. C. Zieman and G. W. Thayer. 1982. Evidence for the influence of seagrasses on the benthic nitrogen cycle in a coastal plain estuary near Beaufort, North Carolina (USA). *Oecologia (Berl.)* 54: 152-158.
- Klump, J. V. and C. S. Martens. 1983. Benthic nitrogen regeneration. In E. J. Carpenter and D. G. Capone (eds.), *Nitrogen in the marine environment*. Academic Press, New York.
- Klump, J. V. and C. S. Martens. 1987. Biogeochemical cycling in an organic-rich coastal marine basin. 5. Sedimentary nitrogen and phosphorus budgets based upon kinetic models, mass balances, and the stoichiometry of nutrient regeneration. *Geochim. Cosmochim. Acta* 51: 1161-1173.
- Koike, I. and A. Hattori. 1978. Simultaneous determinations of nitrification and nitrate reduction in coastal sediments by a ^{15}N dilution technique. *Appl. Environ. Microbiol.* 35: 853-857.
- Koike, I. and J. Sorensen. 1988. Nitrate reduction and denitrification in marine sediments. In T. H. Blackburn and J. Sorensen (eds.), *Nitrogen cycling in coastal marine environments*. John Wiley and Sons, New York.
- Lalli, C. M. and T. R. Parsons. 1993. *Biological oceanography: An introduction*. Pergamon Press, Tarrytown.

- Malan, D. E. and A. McLachlan. 1991. *In situ* benthic oxygen fluxes in a nearshore coastal marine system: A new approach to quantify the effect of wave action. *Mar. Ecol. Prog. Ser.* 73: 69-81.
- McCaffrey, R. J., A. C. Meyers, E. Davey, G. Morrison, M. Bender, N. Luedtke, D. Cullen, P. Froelich and G. Klinkhammer. 1980. The relation between pore water chemistry and benthic fluxes of nutrients and manganese in Narragansett Bay, Rhode Island. *Limnol. Oceanogr.* 25: 31-44.
- Miller-Way, T. and R. R. Twilley. 1996. Theory and operation of continuous flow systems for the study of benthic-pelagic coupling. *Mar. Ecol. Prog. Ser.* 140: 257-269.
- Nixon, S. W. 1981. Remineralization and nutrient cycling in coastal marine ecosystems. In B. J. Neilson and L. E. Cronin (eds.), *Estuaries and Nutrients*. Humana, Clifton.
- O'Neill, J. M. and D. G. Capone. 1989. Nitrogenase activity in tropical carbonate marine sediments. *Mar. Ecol. Prog. Ser.* 56: 145-156.
- Orth, R. J. 1977. The importance of sediment stability in seagrass communities. In B. C. Coull (ed.), *Ecology of marine benthos*. Univ. S. Carolina Press, Columbia.
- Overnell, J., A. Edwards, B. E. Grantham, S. M. Harvey, K. J. Jones, J. W. Leftley and D. J. Smallman. 1995. Sediment-water column coupling and the fate of the spring phytoplankton bloom in Loch Linnhe, a Scottish fjordic sea-loch. Sediment processes and sediment-water fluxes. *Estuar. Coast. Shelf Sci.* 41: 1-19.
- Parsons, T. R., Y. Maita and C. M. Lalli. 1984. *A manual of chemical and biological methods for seawater analysis*. Pergamon Press, Oxford.
- Propp, M. V., V. G. Tarasoff, I. I. Cherbadgi and N. V. Lootzik. 1980. Benthic-pelagic oxygen and nutrient exchange in a coastal region of the Sea of Japan. In K. R. Tenore and B. C. Coull (eds.), *Marine Benthic Dynamics*. University of South Carolina Press, Columbia.
- Puget Sound Estuary Program. 1990 (revised). *Recommended protocols and guidelines for measuring selected environmental variables in Puget Sound*. U. S. Environmental Protection Agency, Region 10, Seattle, Washington. (looseleaf)
- Reddy, K. R., M. M. Fisher and D. Ivanoff. 1996. Wetlands and aquatic processes: resuspension and diffusive flux of nitrogen and phosphorus in a hypereutrophic lake. *J. Environ. Qual.* 25: 363-371.

- Reddy, K. R., R. E. Jessup and P. S. C. Rao. 1988. Nitrogen dynamics in a eutrophic lake sediment. *Hydrobiologia* 159: 177-188.
- Reddy, K. R., W. H. Patrick, Jr. and C. W. Lindau. 1989. Nitrification-denitrification at the plant root-sediment interface in wetlands. *Limnol. Oceanogr.* 34: 1004-1013.
- Risgaard-Petersen, N., S. Rysgaard, L. P. Nielsen and N. P. Revsbech. 1994. Diurnal variation of denitrification and nitrification in sediments colonized by benthic microphytes. *Limnol. Oceanogr.* 39: 573-579.
- Seitzinger, S. P. 1988. Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol. Oceanogr.* 33: 702-724.
- Sherr, E. B. and B. F. Sherr. 1994. Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. *Microb. Ecol.* 28: 223-235.
- Short, F. T. 1983. The response of interstitial ammonium in eelgrass (*Zostera marina* L.) beds to environmental perturbations. *J. Exp. Mar. Biol.* 68: 195-208.
- Sloth, N. P., H. Blackburn, L. S. Hansen, N. Risgaard-Petersen and B. Aa. Lomstein. 1995. Nitrogen cycling in sediments with different organic loading. *Mar. Ecol. Prog. Ser.* 116: 163-170.
- Smith, R. D., A. M. Pregnall and R. S. Alberte. 1988. Effects of anaerobiosis on root metabolism of *Zostera marina* (eelgrass): implications for survival in reducing sediments. *Mar. Biol.* 98: 131-141.
- Sundbäck, K., V. Enoksson, W. Granéli and K. Pettersson. 1991. Influence of sublittoral microphytobenthos on the oxygen and nutrient flux between sediment and water: A laboratory continuous-flow study. *Mar. Ecol. Prog. Ser.* 74: 263-279.
- Sundbäck, K. and W. Granéli. 1988. Influence of microphytobenthos on the nutrient flux between sediment and water: A laboratory study. *Mar. Ecol. Prog. Ser.* 43: 63-69.
- Turner, D. O. 1980. Investigation of the tidal soils of Padilla Bay. Wash. State Univ. Coll., Agric. Res. Cent. Bull. 0885. 15 pp.
- Valiela, I. 1983. Nitrogen in salt marsh ecosystems. In E. J. Carpenter and D. G. Capone (eds.), *Nitrogen in the marine environment*. Academic Press, New York
- Valiela, I. 1984. *Marine ecological processes*. Springer-Verlag, New York.

- Vogel, S. 1983. Life in moving fluids. Princeton University press, Princeton.
- Webber, H. H., T. F. Mumford and J. Eby. 1987. Remote sensing inventory of the seagrass meadow of the Padilla Bay National Estuarine Research Reserve: Areal extent and estimation of biomass. NOAA Tech. Rep. Series OCRM/MEMD. U.S. Dept. Commerce, Washington, D.C. 70 pp.
- Williams, S. L and M. H. Ruckelshaus. 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. Ecology 74: 904-918.
- Yamada, H. and M. Kayama. 1987. Liberation of nitrogenous compounds from bottom sediments and effect of bioturbation by small bivalve, *Theora lata* (Hinds). Estuar. Coast. Shelf Sci. 24: 539-555.
- Zeitzschel, B. 1980. Sediment-water interactions in nutrient dynamics. In K. R. Tenore and B. C. Coull (eds.), Marine Benthic Dynamics. University of South Carolina Press, Columbia.
- Ziebis, W., M. Huettel and S. Forster. 1996. Impact of biogenic sediment topography on oxygen fluxes in permeable seabeds. Mar. Ecol. Prog. Ser. 140: 227-237.

APPENDIX A

Analysis of variance tables for ammonium (NH_4^+), nitrite+nitrate (NO_3^-), and particulate nitrogen (PN) with filtered and unfiltered treatments.

A1. Four-way analysis of variance for ammonium (NH_4^+).

Source of variation	df	F	p
Main effects			
Site	1	5.94	0.0163
Treatment	1	0.33	0.5660
Time	4	6.43	0.0001
Origin	1	528.37	0.0000
Interaction terms			
Site x Treatment	1	0.01	0.9334
Site x Time	4	3.83	0.0059
Treatment x Time	4	0.66	0.6236
Site x Origin	1	0.30	0.5830
Treatment x Origin	1	0.78	0.3797
Time x Origin	4	1.25	0.2934
Site x Treatment x Time	4	1.67	0.1601
Site x Treatment x Time x Origin	13	0.73	0.7281
Error	120		
<hr/>			
Total	159		

A1. Four-way analysis of variance for nitrite+nitrate (NO₃⁻).

Source of variation	df	F	p
Main effects			
Site	1	0.57	0.4514
Treatment	1	0.20	0.6526
Time	4	6.65	0.0001
Origin	1	187.78	0.0000
Interaction terms			
Site x Treatment	1	0.10	0.7570
Site x Time	4	7.16	0.0000
Treatment x Time	4	1.91	0.1112
Site x Origin	1	0.21	0.6462
Treatment x Origin	1	0.28	0.5970
Time x Origin	4	7.18	0.0000
Site x Treatment x Time	4	1.55	0.1899
Site x Treatment x Time x Origin	13	3.39	0.0002
Error	120		
Total	159		

A1. Three-way analysis of variance for particulate nitrogen

Source of variation	df	F	p
Main effects			
Site	1	94.08	0.0000
Treatment	1	0.03	0.8748
Time	4	0.79	0.5394
Interaction terms			
Site x Treatment	1	0.00	0.9962
Site x Time	4	3.00	0.0251
Treatment x Time	4	0.67	0.6165
Site x Treatment x Time	4	1.41	0.2414
Error	60		
Total	79		

APPENDIX B

Analysis of variance tables for ammonium (NH₄⁺), nitrite+nitrate (NO₃⁻), and particulate nitrogen (PN) with filtered and unfiltered treatments pooled.

B1. Three-way analysis of variance for ammonium (NH₄⁺).

Source of variation	df	F	p
Main effects			
Site	1	4.62	0.0334
Time	4	6.92	0.0001
Origin	1	1381.41	0.0000
Interaction terms			
Site x Time	4	2.10	0.0825
Site x Origin	1	1.10	0.2960
Time x Origin	4	3.50	*0.0095
Site x Time x Origin	4	0.81	0.5258
Error	138		
Total	157		

* Significant two-way interaction present; simple main effects evaluated using general contrasts.

B2. Three-way analysis of variance for nitrite+nitrate (NO₃⁻).

Source of variation	df	F	p
Main effects			
Site	1	0.37	0.5424
Time	4	6.45	0.0001
Origin	1	176.83	0.0000
Interaction terms			
Site x Time	4	6.44	0.0001
Site x Origin	1	0.15	0.7011
Time x Origin	4	6.92	0.0001
Site x Time x Origin	4	6.74	*0.0001
Error	138		
Total	157		

* Significant three-way interaction present; simple main effects evaluated using general contrasts.

B3. Two-way analysis of variance for particulate nitrogen.

Source of variation	df	F	p
Main effects			
Site	1	135.72	0.0000
Time	4	0.20	0.9339
Interaction terms			
Site x Time	4	3.51	*0.0115
Error	69		
<hr/>			
Total	78		

* Significant two-way interaction; simple main effects evaluated using general contrasts.

