ATTACHMENT 8

Town of Marion
Two Spring Street
Marion, Massachusetts 02738

November 13, 2015

Ms. Robin Johnson
U.S. Environmental Protection Agency – Region 1
5 Post Office Square, Suite 100 (OEP06-1)
Boston, MA 02109-3912

Mr. David Ferris
Massachusetts Department of Environmental Protection
Wastewater Management Program
1 Winter Street
Boston, MA 02108

Subject: Town of Marion NPDES Permit Supplemental Comments and Activities Update

Dear Ms. Johnson and Mr. Ferris:

The Town of Marion hereby submits the attached letter from our consulting engineers, CDM Smith, which includes the following:

- Supplemental comments related to its Draft NPDES Permit issued by the EPA on November 28, 2014;
- Initial information on an affordability analysis;
- Proposed Total Phosphorus compliance schedule as requested at our recent meeting on November 4, 2015; and
- An update on ongoing planning activities.

Should you have any questions, please feel free to contact me at (508) 748-3550

Sincerely,

Paul F. Dawson
Town Administrator

cc: Mr. Ken Moraff, EPA
        Mr. David Webster, Chief, Water Permits Branch, EPA
        Mr. David Burns, MassDEP
        Ms. Beth Card, MassDEP
        Mr. Doug Fine, MassDEP
        Marion Board of Selectmen
        Mr. Robert Zora, Marion DPW Superintendent
        Mr. Jonathan Witten, Esq., Marion Town Counsel
        Bernadette Kolb, Shawn Syde, Robert Otoski, CDM Smith
        Mark Rasmussen, Buzzards Bay Coalition

November 13, 2015

Ms. Robin Johnson
U.S. Environmental Protection Agency – Region 1
5 Post Office Square, Suite 100 (OEP06-1)
Boston, MA 02109-3192

Subject: Additional Comments on Marion's Draft NPDES Permit

Dear Ms. Johnson:

On behalf of the Town of Marion (Town), this letter contains supplemental comments relating to
Marion's draft National Pollution Discharge Elimination System (NPDES) permit (Draft Permit)
issued on November 28, 2014. The Town requests that the information in this letter be considered
as additional comments to the Town's original response, submitted on February 13, 2015, as well as
supplemental comments submitted on September 16, 2015 and on September 23, 2015. The letter
also includes some initial information to begin the affordability analysis, a proposed total
phosphorus compliance schedule and an update on the activities currently underway on the
lagoons, eelgrass, watershed nitrogen loads, and potential outfall discharges.

Additional Comments on Draft Permit

Seasonal Average Nutrient Limits

The proposed nutrient limits in the Draft Permit are on an average monthly basis. The Town notes
that the recently issued Taunton Wastewater Treatment Plant permit (MA0100030) uses a rolling
seasonal average nutrient limit, recognizing that the nitrogen load over the entire growing season is
more important than the nitrogen load in any given month. Marion requests that the permit limits
in its permit be changed to a rolling seasonal average basis.

The Draft Permit states that the total nitrogen, total phosphorus, and dissolved oxygen seasonal
limits will be in effect from April 1 – October 31. We request that the basis for the seasonal limit be
changed to be in effect between May 1 – October 31. This is consistent with the recently issued
Taunton Wastewater Treatment Plant permit, and is also consistent with the seasonal ammonia
nitrogen limits in the Marion Draft Permit.

Attenuation of Nitrogen in Groundwater

The Draft Permit, relying in part on the study *Environmental Assessment of the Marion Wastewater
Treatment Plant Sewage Lagoons* prepared by the Horsley Witten Group, states that the unlined
lagoons are the largest contributor of nitrogen load to Aucoot Cove. The Town's comment letter
dated February 13, 2015 discussed a myriad of fundamental flaws in the Horsley Witten Group's

estimates of flow and nitrogen transport from the lagoons into the groundwater and ultimately into Aucoot Cove, but may have not adequately emphasize all the pathways to reduction of nitrogen available to any nitrogen that may emanate from the lagoons.

The analysis in the Draft Permit erroneously assumes that all nitrogen discharged from the lagoons ultimately makes it into Aucoot Cove without any attenuation. This is an overly conservative assumption, as significant attenuation of bioavailable nitrogen in groundwater will occur as:

- Impounded water in the lagoons will undergo transformations as influent is aerated (TKN --> ammonia ---> nitrate) and interacts with the low to no oxygen waters at the bottom of the lagoons (denitrification); and

- Groundwater flows through the salt marsh into Aucoot Cove (see attached Valiela and Teal, 1979). Similarly, attenuation will also occur as groundwater passes through shallow marine sediments in Inner Aucoot Cove (see, *e.g.,* Seitzinger, 1988, attached).

Therefore, it is inappropriate to use the estimated load rate to set the allowable nitrogen load to Aucoot Cove necessary to prevent impairment.

Affordability

The proposed conditions in the Draft Permit – especially those relating to the lagoon provisions and the more stringent nutrient limitations – will require significant capital expenditure to be in compliance. With only 1,646 sewer ratepayers who would need to bear the costs associated with these upgrades and significant projected costs of improvements to meet the requirements of the draft NPDES permit, Marion believes the improvements will place the Town above the affordability threshold, and thus subject to regulatory relief as allowed under the Clean Water Act. The Town has not yet completed a detailed affordability analysis but provides the following high-level information to demonstrate the high probability of exceeding the affordability threshold. The Town is undertaking a more detailed affordability analysis and will forward the results when this is completed.

The median household income (MHI) in Marion is $80,456 (see Attachment 3) based on 2013 census data. This MHI is based on all residents within the community; however, it should be noted that not all residents in Marion are connected to the sewer system. Based on the location of the sewered parcels within Town, many of the more affluent portions of Town that drive up the MHI are not connected to the sewer system. As such, it is expected that the MHI of the Town's sewer ratepayers is much less than the Census Bureau's estimated $80,456. Unfortunately, Marion has only one census tract, and we are currently exploring other analyses to determine if it will be possible to refine the MHI to reflect (or at least better reflect) that of the sewer ratepayers.

The Town of Marion estimates that the average household sewer bill is currently about $997 per year, based on a fixed quarterly fee of $104.55 and a tiered billing system based on water consumption. The estimated average bill was developed from actual metered water use data (AMR data) from the Town's MUNIS billing system. Using an existing rate model that accounts for existing debt service, expenditures, O&M and staffing costs, the estimated costs of projects required to meet the conditions in the Draft Permit and other required MS4 expenses, the Town projects that sewer rates will increase by 269%. This increase would mean that the average household sewer bill is projected to increase to $2,683, which is approximately 3.3% of the MHI; significantly above the 2% EPA screening criteria

Total Phosphorus Compliance Schedule

During a November 4, 2015 meeting with EPA and DEP, the regulatory agencies requested that the Town provide further input on the compliance schedule for meeting the total phosphorus limits in the Draft Permit. Our comments start with a copy of the comments on the proposed phosphorus limit related to needed facilities to comply with the limit included in the Town's original letter (page 24) to EPA/DEP (February 13, 2015) on the Draft Permit, and then address potential compliance schedule milestones.

> *Footnote 9 (Page 4 of the Draft Permit) references the compliance schedule for meeting the proposed total phosphorus (TP) limit of 0.2 mg/l and establishes an interim limit from April to October of 1 mg/l. The logic provided in the Fact Sheet for the duration of the compliance schedule is flawed. The schedule assumes that the only WWTF upgrade needed to meet the proposed total phosphorus limit is the addition of chemical storage and dosing facilities. EPA believes 24 months allows sufficient time to evaluate, jar test, and pilot these facilities. Additional upgrades will be needed to meet this limit and include: rapid-mix facilities (potentially, if testing indicates rapid mixing is required), some modification to the filters themselves, and new sludge handling facilities. The need for the sludge handling facilities arises because use of a chemical for phosphorus precipitation will create a chemically-laden (non-biodegradable) sludge that will need to be processed on site and held for off-site disposal.*

> *Phosphorus levels in the treated effluent from September 2010 to August 2014 averaged 1.6 mg/l and ranged from 0.54 to 3.79 mg/l. The current plant, without chemical addition facilities and associated improvements, cannot meet the proposed interim limit of 1 mg/l. Given that the Town will be unable to change its treatment processes to reduce phosphorus levels prior to constructing any upgrades, it is completely unreasonable to select an interim limit of 1 mg/l knowing that this limit could cause the discharge to be immediately out of compliance with the permit....*

On page 36 of the February 13, 2015 letter, the Town provided the following information on the proposed compliance schedule. The information describes the duration of time that the Town

anticipates will be required to complete needed work. Again, text from the February 13, 2015 letter:

> *The Town has reviewed the proposed compliance schedule for actions that the permit mandates (and not the alternatives that the Town thinks need to be considered) and requests revisions to the compliance schedule for these items as follows:* [NB only those related to phosphorus are included, which necessarily include sludge facilities as a chemically laden sludge cannot be discharged to the lagoons in a sustainable manner]

Table 3: Suggested NPDES Permit Compliance Schedule

Permit Section	NPDES Permit Item	Draft Deadline	Suggested Deadline
F.1	Report on Lagoon/Aucoot Cove Compliance	12 months	18 months
F.3	Facilities Plan Amendment	12 months	24 months
F.3	Evaluation/Facilities Plan on TN, TP Limits	12 months	24 months
F.4	Comply with TP Limit (Design/Construction)	24 months	42 months
F.6.a	Progress Report on Lagoons/Sludge Handling	24 months	42 months
F.6.b	Complete Lagoon Liner or Alt. Sludge Handling	36 months	60 months
F.6.b	Comply with Lagoon Requirements (Sludge Management Facilities Design/Construction)	48 months	72 months

* Notes –The schedule also assumes timely review and approval of documents by the regulatory agencies.

In the February 13, 2015 letter, the Town indicated that it believed a different plan (page 36-37) should be followed. This plan included: "Subject the planned improvements to EPA's affordability guidelines and then seek agreement on an implementation schedule that matches these guidelines."

The compliance schedule described in the Town's February 13th comments on the Draft Permit request a 24- month deadline for the evaluation and facilities plan related to the TP limits, and a 42-

month deadline to comply with the TP limit. The Town notes that the schedule proposed in its comment letter represented the minimum feasible compliance schedule (as understood at that time), and that these dates did not take into account additional time that will be needed based on the anticipated start date, the need for the Town to vote for funding for design and construction of these projects at Town Meeting and State Revolving Fund (SRF) program deadlines.

A more detailed schedule concerning actions that might be taken to line the existing lagoons was developed and submitted to the regulatory agencies on September 23, 2015. The schedule in the letter covered 55 months starting with the vote at a Town meeting (no sooner than April 2016) to fund design of the lagoon liner through substantial completion.

With the Town's affordability constraints (see previous comment), additional time will be required to meet the TP conditions described in the Draft Permit. While the final schedule would be based on the forthcoming more detailed affordability analysis, it seems clear at this time that the start of facilities related to phosphorus/sludge handling would need to be delayed until after the completion of the lagoon lining. We would envision a schedule as follows (again from the date that Town Meeting voted affirmatively to support project funding):

- Month 1 (assumed to be May) – Town Meeting, funds appropriated for planning and design

- Month 3 (assumed to be July) – Funds available to start work on TP and sludge processing facilities planning and design

- Month 15 – Complete facilities planning on TP and sludge processing facilities

- Month 21 – Submit draft preliminary design report to EPA/DEP

- Month 23 – Submit final preliminary design report

- Month 27 – Submit 60% plans and specifications

- Month 27 – Begin permitting process

- Month 28 – Submit PEF for SRF funding for construction

- Month 31– Begin public hearings leading up to Town Meeting

- Month 32 – Complete final design, including cost estimate

- Month 37 (typically May) – Town meeting article to fund construction

- Month 39 – Submit SRF loan application with Town appropriation for construction improvements

- Month 40 – DEP issues permission to advertise and project permits in place

- Month 42 – Open bids

- Month 43 – Award construction contract

- Month 45 – Begin construction

- Month 63 – Substantial completion on construction

- Month 65 – Start up period for new facilities prior to permit limits being effective

Update on Planning Activities

The Town is working towards completion of the studies described in the September 15, 2015 letter. The studies include a watershed loading analysis of Aucoot Cove, an analysis of the lagoon water budget and lagoon alternatives, eelgrass analysis, and ocean outfall analysis. Work completed since the previous update includes:

- Completion of 3-months of lagoon depth and flow data collection and refinement of the water balance model; though meters remain in place to year end

- Approximately 180 sludge depth measurements were taken across the three lagoons. Lagoon 1 sludge depth ranged from 6 to 42 inches. Lagoon 2 sludge depth ranged from 2 to 19 inches. Lagoon 3 sludge depth ranged from 2 to 11 inches. The sludge volume in each lagoon is being calculated based on these measurements.

- Two sludge samples were collected per lagoon and analyzed for VOCs, SVOCs, PCBs, MCP metals, total solids, and hazardous waste characterization. TCLP testing is being conducted on some samples. Some analytical results have not yet been received from the lab.

- Issued contract to UMass-Dartmouth to conduct eelgrass field work.

- Began data entry of water use data from the Town to use in the watershed loading analysis, and possibly affordability analysis.

- Continued work on the engineering feasibility of constructing an outfall pipe to the head of the salt marsh in Aucoot cove or constructing an ocean outfall discharging to outer Aucoot Cove.

We expect to complete these studies and submit a draft report to the Town describing the results in February 2016.   A final report is still anticipated in March 2016.

Sincerely,

Bernadette Kolb
Senior Vice President
CDM Smith Inc.

cc: Mr. Ken Moraff, EPA
Mr. David Webster, Chief, Water Permits Branch, EPA
Mr. David Burns, MassDEP
Ms. Beth Card, MassDEP
Mr. Doug Fine, MassDEP
Marion Board of Selectmen
Mr. Paul Dawson, Marion Town Administrator
Mr. Robert Zora, Marion DPW Superintendent
Mr. Jonathan Witten, Esp., Marion Town Counsel
Mr. Shawn Syde, CDM Smith
Mr. Robert Otoski, CDM Smith
Mr. Mark Rasmussen, Buzzards Bay Coalition

Attachments:

1. Seitzinger, S. P. (1988). Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol. Oceanogr.* **33**(4, part 2), 702-724.

2. Valiela, I. and Teal, J.M (1979). The nitrogen budget of a salt marsh ecosystem. *Nature* **280**, 652-656.

3. U.S. Census Bureau, American Fact Finder. Income in the Past 12 Months from the 2009-2013 American Community Survey for the Town of Marion.

Attachment 1

Seitzinger, S. P. (1988). Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance. *Limnol. Oceanogr.* **33**(4, part 2), 702-724.

Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance

Sybil P. Seitzinger
Philadelphia Academy of Natural Sciences, Division of Environmental Research,
Philadelphia, Pennsylvania 19103

Abstract

Denitrification occurs in essentially all river, lake, and coastal marine ecosystems that have been studied. In general, the range of denitrification rates measured in coastal marine sediments is greater than that measured in lake or river sediments. In various estuarine and coastal marine sediments, rates commonly range between 50 and 250 μmol N m^{-2} h^{-1}, with extremes from 0 to 1,067. Rates of denitrification in lake sediments measured at near-ambient conditions range from 2 to 171 μmol N m^{-2} h^{-1}. Denitrification rates in river and stream sediments range from 0 to 345 μmol N m^{-2} h^{-1}. The higher rates are from systems that receive substantial amounts of anthropogenic nutrient input. In lakes, denitrification also occurs in low oxygen hypolimnetic waters, where rates generally range from 0.2 to 1.9 μmol N liter^{-1} d^{-1}. In lakes where denitrification rates in both the water and sediments have been measured, denitrification is greater in the sediments.

The major source of nitrate for denitrification in most river, lake, and coastal marine sediments underlying an aerobic water column is nitrate produced in the sediments, not nitrate diffusing into the sediments from the overlying water. During the mineralization of organic matter in sediments, a major portion of the mineralized nitrogen is lost from the ecosystem via denitrification. In freshwater sediments, denitrification appears to remove a larger percentage of the mineralized nitrogen. N$_2$ fluxes accounted for 76–100% of the sediment–water nitrogen flux in rivers and lakes, but only 15–70% in estuarine and coastal marine sediments. Benthic N$_2$O fluxes were always small compared to N$_2$ fluxes.

The loss of nitrogen via denitrification exceeds the input of nitrogen via N$_2$ fixation in almost all river, lake, and coastal marine ecosystems in which both processes have been measured.

Denitrification is also important relative to other inputs of fixed N in both freshwater and coastal marine ecosystems. In the two rivers where both denitrification measurements and N input data were available, denitrification removed an amount of nitrogen equivalent to 7 and 35% of the external nitrogen loading. In six lakes and six estuaries where data are available, denitrification is estimated to remove an amount of nitrogen equivalent to between 1 and 36% of the input to the lakes and between 20 and 50% of the input to the estuaries.

Denitrification is carried out by many heterotrophic, generally facultative anaerobic bacteria. These bacteria utilize nitrite or nitrate as the terminal electron acceptor during the oxidation of organic matter and produce N$_2$, NO, or N$_2$O (Payne 1973):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2.$$

Denitrification has ecological and geochemical consequences in both freshwater and coastal marine systems. Phytoplankton production in numerous coastal marine areas (Ryther and Dunstan 1971) as well as a number of lakes (Keeney 1973) has been identified as limited by nitrogen, or by both nitrogen and phosphorus (Gerhart and Likens 1975). The removal of fixed nitrogen can be important, therefore, in regulating the amount of primary production in such systems. In waters that receive substantial amounts of anthropogenic nutrients, denitrification may help control the degree of eutrophication. Denitrification is a sink in the global marine nitrogen budget, and, as discussed below, denitrification in estuaries decreases the amount of continentally derived nitrogen transported to the oceans.

Several reviews have addressed the biochemistry and physiology of denitrification (Painter 1970; Payne 1973; Focht and Verstraete 1977; Knowles 1982), the rates found in marine (Knowles 1982; Hattori 1983) or freshwater systems (Knowles 1982), and denitrification in general (Delwiche and Bryan 1976; Delwiche 1981). In this review, I discuss and compare the rates of denitrification reported for streams and rivers, lakes, and subtidal coastal marine ecosystems. I then compare the magnitude of denitrification to other nitrogen processes in those systems, including nitrogen mineralization rates in the sediments, N$_2$-fixation rates, and exter-

nal nitrogen inputs to those systems. The effect of denitrification on the N : P ratio in those systems and the factors controlling denitrification rates also are discussed.

Methods used to measure denitrification

Various methods are used to measure denitrification. Most of them are indirect, partly due to the large background concentration of N_2 in the atmosphere and dissolved in water which makes it difficult to detect an increase in N_2 concentration from denitrification in a natural system. Several detailed reviews of denitrification methodology are available (Knowles 1982; Taylor 1983), but a brief discussion of the more commonly used methods is necessary since interpretation of the rates reported in the literature is partly dependent on the methods that were used.

The mass balance approach has frequently been used to estimate denitrification rates in lakes (Ahlgren 1967; Andersen 1971; Serruya 1975; Likens and Loucks 1978; Messer and Brezonik 1983) and rivers (Owens et al. 1972; Kaushik and Robinson 1976; Thomson 1979; Hill 1979, 1981, 1983). The external inputs of N from sources such as streams, seepage, runoff, precipitation, and, in some cases, nitrogen fixation are quantified, and the removal of N by burial in the sediments and by washout are determined; the difference is attributed to denitrification. While such studies may indicate the scale of denitrification, the occurrence and magnitude of any process estimated from imbalances in whole ecosystem nitrogen budgets is subject to considerable error (i.e. the combined error in each of the input and removal terms). Mass balance studies also do not provide information on the site of denitrification (sediments or water column), spatial variability, or the factors controlling the process. However, if carried out over one or more annual cycles, this approach has the advantage of providing an integrated, system-scale estimate of denitrification.

The rate of decrease in the nitrate or nitrite concentration in water over sediment cores has often been used to estimate denitrification rates (Andersen 1977; Robinson et al. 1979). (Hereafter, nitrate will be used to designate both nitrite and nitrate,

unless otherwise noted.) This approach may overestimate denitrification rates because nitrate also can be reduced to ammonium (Koike and Hattori 1978; Sørensen 1978a) or incorporated into organic matter (Chan and Campbell 1980). A modification of this technique, in which $^{15}NO_3^-$ is added to the water overlying the sediments and the rate of $^{15}N_2$ production is measured, avoids these problems (Tiren et al. 1976; Chan and Campbell 1980; Nishio et al. 1983). However, either approach may underestimate denitrification rates since denitrification of nitrate produced in the sediments is not measured and, as discussed below, this is often the major source of nitrate for denitrification. However, by also measuring $^{15}N_2$ production from $^{15}NH_4^+$ added to the overlying water, which diffuses into the sediments and is nitrified, the total denitrification rates can be measured in relatively undisturbed sediments (Nishio et al. 1983; Jenkins and Kemp 1984).

The response of denitrification rates in sediment slurries to increasing nitrate concentrations can often be described by Michaelis-Menten-type kinetics. When combined with measurements of the vertical distribution of nitrate in pore waters, such relationships have been used to estimate in situ denitrification rates (Vanderborght and Billen 1975; Madsen 1979; Oren and Blackburn 1979). However, the use of such a relationship to estimate in situ sediment denitrification rates in the field is not straightforward. For example, laboratory measurements indicate that the relationship between denitrification rate and nitrate concentration can vary with depth in the sediment (Kaspar 1983). This was also demonstrated in Danish coastal sediments where maximum denitrification rates with depth did not correlate with maximum NO_3^- concentrations (Sørensen 1978b). In sediments from Kenepura Sound, New Zealand, no relationship was observed between depth profiles of ambient NO_3^- concentrations and denitrification rates (Kaspar et al. 1985b). The relationship between denitrification rate and NO_3^- concentration has also been found to vary between sites. For example, while nitrate in the sediments at two different sites in Kenepuru Sound was similar, denitrifi-

cation rates differed by a factor of seven (Kapsar et al. 1985b). Similarly, at nine locations off the west coast of New Zealand, nitrate concentrations varied by a factor of two among sites, while denitrification rates varied by over a factor of 10 (Kaspar et al. 1985a).

The discrepancy between field and laboratory measurements may be, in part, one of scale. The nitrate concentration in microenvironments may be controlling denitrification rates; not the bulk nitrate concentration as measured at 1-cm depth intervals. Additional factors discussed below may also influence denitrification.

The finding that acetylene blocks the reduction of N_2O to N_2 (Balderston et al. 1976; Yoshinari and Knowles 1976) and the availability of sensitive gas chromatographic methods for measuring N_2O have led to a relatively easy and rapid technique for measuring denitrification rates. In addition, depth profiles of denitrification can be measured when this technique is used on whole cores (e.g. Sørensen 1978b; Kaspar 1982; Oremland et al. 1984). However, acetylene also inhibits nitrification (Hynes and Knowles 1978). Therefore, in sediments where nitrate concentrations are low and there is a rapid coupling between denitrification and nitrification, denitrification rates will likely be underestimated. At low nitrate concentrations, acetylene incompletely blocks N_2O reductase which, in some cases, may be countered by using high acetylene concentrations (Kaspar 1982; Oremland et al. 1984). Sulfide reverses acetylene blockage of N_2O reductase (Tam and Knowles 1979), which may be particularly problematic in highly organic marine sediments. Additional problems associated with the rate of diffusion of acetylene into whole cores have been discussed by Oremland et al. (1984).

The direct measurement of denitrification as N_2 production from intact sediment cores incubated in gastight chambers (Seitzinger et al. 1980) has been used in coastal marine, lake, and river sediments. The sediment cores are kept under natural conditions of nitrate and oxygen concentrations in the overlying water. The background N_2 concentration is reduced by sparging the water and gas phase over the sediments with a mixture of 21% oxygen, 0.03% CO_2, and the balance helium. Lowering the background N_2 concentration allows measurement of N_2 increases due to denitrification. The advantage of this technique is that it is a direct measurement of denitrification and does not use inhibitors, or alter the nitrate or oxygen concentrations. This technique also measures denitrification of nitrate both from the overlying water and from nitrification in the sediments. One disadvantage is that incubation times of about 9 d are required to deplete the background N_2 concentration in the pore waters. However, repeated measurements of ammonia, nitrate, nitrite, and oxygen sediment–water fluxes indicate that these parameters do not markedly change during that time (Seitzinger 1982), and thus it is likely that denitrification rates also are not markedly changed. Although some have speculated that contamination of samples by atmospheric N_2 may be a problem with this technique, blanks are routinely run (by sampling from helium-flushed chambers without sediments) and demonstrate that, with the sampling procedures outlined by Seitzinger et al. (1980), atmospheric contamination is avoidable.

Denitrification rates in aquatic ecosystems

Rates of denitrification have now been reported for sediments and water in many freshwater and coastal marine ecosystems. For the present discussion, unless noted, I am including only those rates where actual measurements of denitrification were made (not rates inferred from mass balance calculations within an ecosystem) and only rates measured at near-ambient nitrate, organic matter, and oxygen concentrations. Most of these measurements are confined to temperate zones in the northern hemisphere.

In rivers and streams, denitrification has been recognized as a potential pathway of nitrogen loss based on deficiencies in mass balance calculations of nitrate and/or total nitrogen (Owens et al. 1972; Kaushik and Robinson 1976; Thomson 1979; Hill 1979, 1981, 1983). However, few actual measure-

ments of denitrification in sediments of streams or rivers have been reported. Those reported generally range from 54 to 345 μmol N m^{-2} h^{-1} (Table 1). In periphyton from a pristine stream, no denitrification was found (Duff et al. 1984). The high rates are from streams or rivers that receive substantial amounts of anthropogenic nutrients from land runoff or sewage inputs, including Swifts Brook, the Potomac River, and the Delaware River. Seasonal measurements of denitrification in streams and rivers are lacking, and most studies have been made in only a limited section of the river or stream.

No measurements of denitrification in hypoxic or anoxic river water were found, and only one study of denitrification in river sediments exposed to low oxygen waters was found. Denitrification rates were reduced by 83–99% in stream periphyton under aerobic compared to anaerobic conditions (Duff et al. 1984). Low-oxygen conditions are common in rivers that receive large BOD inputs from sewage treatment plant discharges; those same rivers also usually receive large inputs of N. The effect of low oxygen conditions on denitrification rates in both river waters and sediments should be investigated, as well as changes in denitrification rates that occur when low-oxygen conditions are alleviated.

The first direct evidence of denitrification in lake sediments was based on the production of ^{15}N$_2$ from ^{15}NO$_3^-$ added to water from Smith Lake, Alaska, incubated in bottles with and without sediment (Goering and Dugdale 1966). Substantially higher ^{15}N$_2$ production occurred in the samples with sediments. Denitrification rates ranging from 2 to 171 μmol N m^{-2} h^{-1} have been measured at near-ambient conditions in sediments from a variety of lakes, but all except two reported rates are <65 μmol N m^{-2} h^{-1} (Table 1). There is no obvious relationship between the rate of denitrification reported and the trophic status of the lake. However, this may, in part, be due to incomplete measurements. For example, in ELA Lake 227, in Bryrup Langsø, and in Kvind sø, only denitrification of nitrate diffusing into the sediments from the overlying water was measured; denitrification of ni-

trate produced in the sediments was not detected by the techniques employed. In Lake Mendota, only denitrification of nitrate from groundwater input was estimated, and not measured. As discussed below, nitrification in the sediments is often a major source of nitrate for denitrification.

The most extensive measurements of denitrification in lake sediments are from Lake Okeechobee, Florida (Messer and Brezonik 1983), Lake Michigan (Gardner et al. 1987), and Lake Hampen (Christensen and Sørensen 1986). In Lake Okeechobee, denitrification was measured at two locations and on three occasions during winter and spring using the acetylene inhibition technique with sediment slurries. In southeastern Lake Michigan, denitrification rates were measured over an annual cycle at two locations as a flux of N$_2$ from intact sediment cores. In Lake Hampen, denitrification rates were measured seven times over a 17-month period at one location in vegetated littoral sediments. Other denitrification studies in lake sediments have been made at only one time of year, at one location, or with techniques that likely measured only a portion of total sediment denitrification.

If sufficient nitrate is available, denitrification can occur in low oxygen hypolimnetic waters in lakes, as well as in sediments. Pelagial denitrification rates for four lakes (Table 2) ranged from 0.2 to 1.9 μmol N liter^{-1} d^{-1} in lakes Mendota, Smith, and ELA 227. Much higher rates were reported for Lake Kinneret (3–36 μmol N liter^{-1} d^{-1}) where nitrate concentrations are also high (up to 107 μM).

In lakes where measurements have been made in both the water and sediments, sediment denitrification is a greater sink for nitrogen than is denitrification in the water column. In Lake Mendota, the amount of denitrification in the sediments (Keeney et al. 1971) is about twice as large as that in the water (Brezonik and Lee 1968). The importance of sediment denitrification in Lake Mendota may be even greater than current data indicate because only nitrate seeping into the sediments from groundwater was considered. In Lake 227, Chan and Campbell (1980) concluded that denitrification in the water column was not a significant N

Table 1. Denitrification rates measured at near-ambient conditions in sediments from rivers, streams, lakes, and coastal marine systems.

Location	Rate (μmol N m^{-2} h^{-1})		Notes*	Reference
River/streams				
San Francisquito Creek, California	54		1	Duff et al. 1984
Little Lost Man Creek	0		1	Duff et al. 1984
Swift Brook, Ontario	121–302†		2	Robinson et al. 1979
Potomac River	210–235		3	Seitzinger 1987a
Delaware River	166–345		4	Seitzinger in press
Lakes				
Oligotrophic/mesotrophic				
Michigan	12–51		5	Gardner et al. 1987
Hampen, Denmark	5–50		6	Christensen and Sørensen 1986
Lacawac, Pennsylvania	50		7	Seitzinger unpubl. data
Ernest, Pennsylvania	56		7	Seitzinger unpubl. data
Moderately eutrophic				
Okeechobee	2–25		8	Messer and Brezonik, 1983
Mendota, Wisconsin	10†		9	Keeney et al. 1971
Arresø	11		10	Madsen 1979
Eutrophic				
ELA 227	42–58†		11	Chan and Campbell 1980
Bryrup Langsø	171†		12	Andersen 1977
Kvind sø	102†		12	Andersen 1977
Coastal marine				
Patuxent R. estuary	77–89		13	Jenkins and Kemp 1984
Izembek Lagoon, Alaska	3.5–34.5		14	Iizumi et al. 1980
Delaware Inlet, N.Z.	6–12		15	Kaspar 1983
Kenepuru Sound, N.Z.	6–35		16	Kaspar et al. 1985b
	28–253			
Tama estuary	145–594		17	Nishio et al. 1983
Odawa Bay, Japan	37		18	Nishio et al. 1983
Tokyo Bay, Japan	16–33†		19	Nishio et al. 1982
Kysing Fjord, Denmark	7		20	Oren and Blackburn 1979
Tejo estuary, Portugal	107–1,067	(232)	21	Seitzinger unpubl. data
Delaware Bay	0–260	(98)	22	Seitzinger unpubl. data
Ochlockonee Bay, Florida	0–210	(75)	23	Seitzinger 1987b
Narragansett Bay, Rhode Island	39–109	(59)	24	Seitzinger et al. 1984
Great Bay, Long Island	3–16		25	Slater and Capone 1987
Randers Fjord	6–42		26	Sørensen 1978b; Sørensen et al. 1979
Kysing Fjord	1–330			
Cosatal North Sea	0–84	(50)	27	Billen 1978
Landrup Vig	12–213		28	Andersen et al. 1984
Four League Bay	2–74		29	Smith et al. 1985
MERL mesocosms	305–888		30	Seitzinger and Nixon 1985
West coast, N.Z.	0.4–141		31	Kaspar et al. 1985a
San Francisco Bay	0.8–1.2		32	Oremland et al. 1984

* 1. Denitrification in undisturbed periphyton communities; acetylene blockage technique; authors reported 651 μmol N$_2$O produced m^{-2} d^{-1} as average light, aerobic plus dark, and anaerobic rates over a 24-h period; summer measurements. 2. Calculated from data derived from authors' figure of rate of decrease in NO$_3$ over sediment cores; lower rate at NO$_3$ concentrations 0.5–2.0 mg NO$_3$-N liter^{-1} and higher rate with NO$_3$ concentrations 2–4 mg NO$_3$-N liter^{-1}, similar to range in ambient stream concentrations; 22°C; role of denitrification confirmed by ^{15}N studies. 3. Measurements made in September 1985; rates from two locations in tidal freshwater portion of river near and in Gunston Cove; direct N$_2$ flux measurements from intact cores. 4. Measurements made in August 1984; range of rates at five locations in the tidal freshwater portion of river between Trenton and Little Tinicum Is.

5. Denitrification measured as flux of N$_2$ from intact sediment cores collected from two sites in southeastern portion of lake; range of seasonal rates. 6. Range of rates measured over an annual cycle; rates measured as N$_2$O production in presence of acetylene in intact cores of vegetated littoral sediment; ambient nitrate concentrations in the sediments ranged from 10 to >200 μM. Lowest rates in winter, highest in summer. 7. Measurements made in September 1985; direct N$_2$ flux measurements from intact cores collected in epilimnion.

8. Acetylene inhibition technique using slurries; range of average annual rates for the whole lake (0.5–1.3 g N m^{-2} yr^{-1}) reported by authors based on measurements made three times during winter and spring. 9. Annual denitrification of groundwater inputs estimated from measurements of difference between rate of decrease in ^{15}NO$_3$$^-$ in sediment slurries and rate of increase in ^{15}NH$_4$$^+$ and ^{15}N-organic N. 10. Annual average denitrification rate calculated according to Vanderborght and Billen 1975 using denitrification rate constant determined from ^{15}N$_2$ production in sediment slurries incubated with 390 μM ^{15}NO$_3$$^-$, and NO$_3$ concentration in lake water throughout year. Does not include denitrification of nitrate produced in sediment.

Table 1. Footnotes continued.

11. Lake artificially enriched with NO_3^- and PO_4^{3-} in summer; denitrification measured as $^{15}N_2$ production from $^{15}NO_3^-$ added to water in dome over epilimnetic sediments; minimum estimate, as does not include denitrification of any NO_3^- produced in sediments; range of two measurements made in September. 12. Annual denitrification rates (1972–1973) calculated by authors from rate of NO_3^- decrease over sediment cores collected in June (Bryrup Langsø) or January (Kvind sø) and from NO_3^- concentration in lakes during the year.

13. April, 17°C, range and average of upper and lower estuary sediments; denitrification measures as $^{15}N_2$ production from sediment cores incubated with $^{15}NO_3^-$ in overlying water. 14. Range of rates reported for muddy, sandy, and *Zostera* bed sediments; 11–15°C; denitrification measured as $^{15}N_2$ production from sediments from various depths incubated with $^{15}NO_3^-$ and calculated in situ rates based on ambient NO_3^- concentrations; rates reported for top 7 cm as g-atoms N g^{-1} h^{-1}; converted to μg-atoms m^{-2} h^{-1} assuming 1.4 g wet sediment cm^{-3} over 7 cm depth. 15. Average rates reported for top 10.5 cm based on N_2O accumulation in undisturbed core segments in presence of acetylene; March and April 1981; rates reported as mg N m^{-2} d^{-1}; author-corrected data for errors imposed by acetylene technique. 16. Rates reported for top 12 cm based on N_2O accumulation in undisturbed core segments in presence of acetylene; January, October 1983; rates reported as mmol N m^{-2} d^{-1}; sediments had epibenthic algae.

17. Muddy fine sand; rates measured as $^{15}N_2$ produced from $^{15}NO_3^-$ and $^{15}NH_4^+$ in water flowing over cores; rates reported as ng-atoms cm^{-2} h^{-1}. 18. Fine sand rich in carbonate; rates measured as in Tama estuary above; rates reported as ng-atoms cm^{-2} h^{-1}. 19. Measured as rate of $^{15}N_2$ from intact sediment cores with 10–20 μM $^{15}NO_3^-$ in water flowing over cores; rates may be underestimated as only denitrification of NO_3^- diffusing into sediments from overlying water and not of NO_3^- produced in sediments was measured; September.

20. Rate measured as $^{15}N_2$ production from $^{15}NO_3^-$ added to sediment slurries; rates calculated for in situ NO_3^- concentration; rates at 12°C.

21. Range (average of all but highest rate) of rates measured at eight locations in bay in November 1983; measured as N_2 production from intact sediment cores. 22. Range of rates measured at eight locations in bay in July and September and average rate for bay during that time; measured as N_2 production from intact sediment cores. 23. Range (annual average) of rates throughout bay measured in 1984–1985; measured as N_2 production from intact sediment cores. 24. Range and average annual rate in bay 1980–1981; measured as N_2 production from intact sediment cores; silt-clay sediments. 25. Range of rates measured throughout year; denitrification measured as rate of N_2O accumulation in presence of acetylene in 2-cm sections of cores from various depths; sandy sediments with high nitrate groundwater intrusion.

26. Sandy sediments; lower rate for each fjord is from June measurements (18°C) and higher rate from January measurements (3°C); rates measured as N_2O production from intact cores incubated with acetylene; rates reported as mmol N m^{-2} d^{-1}. 27. Range of rates calculated from mathematical analysis of pore-water concentration profiles and measured rate of consumption of NO_3^- added to sediment samples; average annual rate for coastal zone. 28. Soft, muddy sediments covered with benthic microalgae; range of rates over 24-h cycle for April and June measurements; measured as N_2O production from intact cores incubated with acetylene; rates reported as mmol N m^{-2} d^{-1}.

29. Range of rates measured at five sites over an annual cycle; rates measured as N_2O production in the presence of acetylene in the top 3 cm of a mixed sediment sample; rates reported as ng N g^{-1} h^{-1} were converted to μmol N m^{-2} h^{-1} assuming 14,789 g dry sediment per m^{-2} which was conversion factor used by authors to calculate annual areal rates. 30. Range of rates measured in September in four estuarine mesocosms receiving a range of nutrient additions (N, P, and Si); highest denitrification rates from sediments in microcosm receiving highest rate of N loading; denitrification rates measured as N_2 production from intact sediment cores. 31. Range of rates reported for near shore sediments at five locations at room temperature in January; denitrification rates measured as rate of N_2O accumulation in presence of acetylene in 1.5-cm sections of the top 7 cm of sediment; rates corrected by authors for errors imposed by acetylene technique.

32. Range of rates for upper 3 cm of intertidal mudflats near sewage treatment discharge site; denitrification measured using acetylene blockage technique at 20°C. Authors state probably underestimate because of inhibition of nitrification and diffusion rate of gases in sediments.

† These rates may be underestimated as technique did not measure dentrification coupled to sediment nitrification.

sink. The larger amount of nitrogen removed by denitrification in sediments relative to denitrification in the water column is not surprising due to the low oxygen conditions, rapid nitrification rates, and abundant supply of organic matter in the sedi-

Table 2. Denitrification rates in the water column of various lakes and the Baltic Sea under low oxygen or anoxic conditions.

Location	Rate (μmol N liter^{-1} d^{-1})	Comments	Reference
Lake Mendota	0.6–1.9	Measurements in hypolimnion in summer; denitrification calculated as difference between NO_3^- disappearance in lake and $^{15}NH_4^+$ and ^{15}N-organic N production from $^{15}NO_3^-$; 7 μM NO_3^-.	Brezonik and Lee 1968
Lake Kinneret	3–36	Measurements made under anaerobic conditions; range of NO_3^- concentrations 7–107 μM; denitrification calculated as change in total N in water sample with time.	Cavari and Phelps 1977
ELA Lake 227	0.2–1.6	Denitrification measured in anoxic hypolimnion as $^{15}N_2$ production from $^{15}NO_3^-$; summer 1974 rates between 4.0 and 4.5 m; DO < 0.2 mg liter^{-1}; 14 μM $^{15}NO_3^-$	Chan and Campbell 1980
Shallow arctic lake	1.1	Denitrification measured in anoxic water as $^{15}N_2$ production from $^{15}NO_3^-$; winter rates; 36 μM $^{15}NO_3^-$.	Goering and Dugdale 1966
Baltic Sea	0.10	Denitrification calculated from nitrate anomalies in the low oxygen waters below the halocline; <10 μM NO_3^-.	Shaffer and Rönner 1984

Table 3. N loading rates and calculated denitrification rates from annual N mass balance studies in various lakes. Rates reported as μmol N m^{-2} h^{-1} for comparison with Table 1.

Lake	N input	Calculated denitrification rate	Removal by denitrification (%)	Reference
Bryrup Langsø				
1972	690	326	47	Andersen 1971
1973	657	326	50	
Kvind sø				
1972	1,427	244	17	Andersen 1971
1973	1,215	260	22	
Kul sø				
1972	897	163	18	Andersen 1971
1973	872	195	22	
Salten Langsø				
1972	216	0	0	Andersen 1971
1973	194	18	10	
Halle sø				
1972	703	383	54	Andersen 1971
1973	696	359	51	
Stigsholm sø				
1972	650	148	22	Andersen 1971
1973	666	171	26	
Norrviken	542	199	37	Ahlgren 1967
Kinneret				
1968–1969	245	152	62	Serruya 1975
1969–1970	74	43	58	
Mirror	12.3	0.4	3	Likens and Loucks 1978
Wingra	192	50	26	Likens and Loucks 1978
Okeechobee	46	8.2	18	Messer and Brezonik 1983

ments. However, additional studies of denitrification in lakes are needed to evaluate further the relative importance of these two sites.

Denitrification rates in several lakes have been inferred from the imbalances in whole lake N budgets (Ahlgren 1967; Andersen 1971; Serruya 1975; Likens and Loucks 1978; Messer and Brezonik 1983). Many of the rates estimated by this technique are greater than those measured more directly (Table 3). In general, the higher rates appear to be from lakes that have high N loading rates.

Direct evidence of denitrification in marine sediments was provided by measurements of $^{15}N_2$ production from $^{15}NO_3^-$ added to continental shelf sediments from the coast of Peru (Goering and Pamatmat 1971). Denitrification rates in many estuarine and coastal areas (Table 1) range from 0 to 1,067 μmol N m^{-2} h^{-1}, with rates commonly reported between 50 and 250 μmol N m^{-2} h^{-1} as estimated with various techniques. The highest have been found in sediments from eutrophic areas: the Tama estuary (up to 594 μmol N m^{-2} h^{-1}), an area of the Tejo estuary (1,067 μmol N m^{-2} h^{-1}) that receives large amounts of sewage, and a marine mesocosm (888 μmol m^{-2} h^{-1}) that received high nutrient loading. Denitrification measurements over an annual cycle have been made in Kysing Fjord (Sørensen 1984), Narragansett Bay (Seitzinger et al. 1984), Four League Bay (Smith et al. 1985), and Ochlockonee Bay (Seitzinger 1987b).

Although increasing numbers of estuaries may be developing hypoxic or anoxic bottom waters as a result of increased eutrophication, denitrification rates in oxygen-deficient waters have been estimated in only

one area. Denitrification rates in the oxygen-deficient zone of the water column (O_2 ~10 μmol liter^{-1}) in the Baltic Sea were estimated to be about 3.6 μmol N liter^{-1} d^{-1} (Shaffer and Rönner 1984). For the Baltic as a whole, 80–90% of the denitrification was estimated to occur in the sediments and only 10–20% in the water.

In general, the range of denitrification rates measured in coastal marine sediments is greater than in lake or river sediments. However, the limited number of total denitrification rate measurements (i.e. denitrification of nitrate diffusing in from the water and that of nitrate produced in the sediments) in lake or river sediments makes conclusions about the relative magnitude of denitrification rates in rivers, lakes, and coastal marine systems uncertain. More useful, perhaps, is a comparison of denitrification rates to other nitrogen processes in those systems.

Sources of nitrate for denitrification

An adequate supply of nitrate is essential to drive the denitrification process. There are three sources of nitrate for sediment denitrification: nitrate diffusing into the sediments from the water column; nitrate produced in the sediments via nitrification of ammonia released from benthic oxidation of organic matter; and nitrate advected through the sediments from groundwater. Few studies have addressed the latter, although it is potentially important, especially in areas where groundwaters are contaminated with nitrate from agricultural, feedlot, industrial, or sewage sources. In Lake Mendota, 63% of the nitrate advected through the sediments in groundwater was estimated to be denitrified (Keeney et al. 1971). In Great Bay, Long Island, groundwater is a source of NO_3^- for denitrification, especially in the 14–40-cm-deep layer of the sediments (Slater and Capone 1987). About 16% of the groundwater NO_3^- in the deeper sandy layers, which are low in organic carbon, is denitrified.

Nitrate produced in the sediments appears to be the major substrate for denitrification in most river, lake, and coastal marine sediments. This conclusion is based on concurrent measurements of denitrification and sediment–water nitrate flux, which show either a net flux of nitrate out of the sediments or a flux of nitrate into the sediments from the water column that is less than the measured denitrification rate (Table 4). For instance, in Gunston Cove, located in the tidal freshwater portion of the Potomac River, the uptake of nitrate from the water by sediments was 107±18 μmol N m^{-2} h^{-1} (mean±SD), while the denitrification rate (N_2 production) was about twice as great, 210±31 μmol N m^{-2} h^{-1} (Seitzinger 1987a). In the mainstem of the Potomac River and in the tidal freshwater portion of the Delaware River, no net uptake of nitrate from the water column by the sediments occurred, although rates of denitrification ranged from 166 to 344 μmol N m^{-2} h^{-1}. Thus, although water column nitrate concentrations were quite high (>70 μM), nitrification in the sediments appears to be the major source of nitrate for denitrification. The same pattern—greater denitrification rates compared to nitrate fluxes into the sediments from the water column—is found in many lake and coastal marine sediments (Table 4). Of course, a net flux of nitrate out of the sediments does not eliminate the possibility that some nitrate from the water column enters the sediments by mechanisms such as bioturbation, irrigation, or diffusion, and is subsequently denitrified. A direct demonstration of the tight coupling between sediment nitrification and denitrification comes from ^{15}N studies using intact sediment cores. In Patuxent River estuary sediments, over 99% of the $^{15}NO_3^-$ formed from $^{15}NH_4^+$ in the sediments was reduced to $^{15}N_2$ during spring experiments (Jenkins and Kemp 1984). Those experiments, however, did not examine the magnitude of denitrification dependent on nitrate from the overlying water column. In contrast to the above studies, nitrate from the overlying water is the major source of nitrate for denitrification in Tama estuary sediments (Nishio et al. 1983).

The importance of nitrification in sediments as a source of nitrate for denitrification is consistent with the high rates of nitrification measured in coastal sediments (Kaplan 1983). It is interesting to note that nitrification rates per unit volume in sediments are at least an order of magnitude greater than nitrification rates in the water

Table 4. Sediment–water fluxes of ammonia, nitrate plus nitrite, and N_2, and percent of N flux due to denitrification [N_2 flux/(NH_4^+ + NO_3^- + NO_2^- + N_2)flux] in river, lake, and coastal marine sediments. Units: μmol N m^{-2} h^{-1}. The major or sole source of nitrate for denitrification in all these systems is from nitrification in the sediments as indicated by the flux of nitrate out of the sediments. If the water column were the major source, there would be a flux of nitrate into the sediments similar in magnitude to the denitrification rate. (Minus indicates uptake.)

Location	NH_4^+	NO_3^- + NO_2^-	N_2	N_2, % of total N flux	Reference[*]
	(μmol N m^{-2} h^{-1})				
Rivers					
Potomac					
Mainstem	15	50	232	78	1
Gunston Cove	3	−107	210	99	1
Delaware River					
Philadelphia area	4	7	344	97	2
Near Mud Grass Island (S of Trenton)	0	0	166	100	2
Lakes					
Lacawac	0	15	50	77	3
Ernest	0	20	56	74	3
Kvind sø	†	†	74	100	4
Michigan					
45-m water depth	0	1	35	97	5
100-m water depth	0	0.3	22	99	5
Coastal marine					
Ochlockonee Bay	26	18	75	63	6
Narragansett Bay	91	10	59	37	7
North Sea, Belgian coast					
Coastal	73	98	49	22	8
Offshore	41	49	16	15	8
North Sea, Belgian coast	92	100	88	31	9
Patuxent estuary	467	−42	133	20	10
Delaware Bay					
Midbay	15	54	93	57	11
Lower bay	11	193	75	27	11
Tejo estuary, Portugal					
Upper bay	28	212	172	42	12
S. Island, west coast, N.Z.					
Sta. R200	17	17	10	23	13
Sta. R212	17	8	77	75	13
Sta. 213	21	25	53	54	13

[*] 1. Seitzinger 1987a; tidal freshwater portion of river mainstem station near Indian Head, October 1985. 2. Seitzinger in press; tidal freshwater portion of river, August 1985.

3. Seitzinger unpubl. data; epilimnetic sediments, October 1985. 4. Andersen 1971, 1977; rates for October 1973, there was a net uptake of ammonia and nitrate from the water column, denitrification for October was calculated from the NO_3^- concentration in the lake in October and the relationship between nitrate uptake by sediments from water and nitrate concentration. 5. Gardner et al. 1987; annual measurements in southeastern portion of lake.

6. Seitzinger 1987b; average of measurements from all cores throughout year. 7. Seitzinger et al. 1984; Nixon et al. 1976; annual average midbay data. 8. Billen 1978; annual average. 9. Vanderborght et al. 1977. 10. Reported in Henriksen and Kemp 1988. 11. Seitzinger unpubl. data; July. 12. Seitzinger unpubl. data; November, muddy sediments from intertidal oyster reef. 13. Kaspar et al. 1985a; ammonia and nitrate fluxes calculated from concentration gradient between top 1.5 cm of sediment pore water and overlying water.

† Reported a combined flux of NH_4^+ + NO_3^- + NO_2^- into the sediment of 36–45 μmol N m^{-2} h^{-1}.

column. For example, nitrification rates in coastal sediments are often 20 μmol liter^{-1} h^{-1}, whereas in coastal waters rates range from only ~0.001 to 0.1 μmol liter^{-1} h^{-1} (Kaplan 1983). Of course, the volume of sediments in which nitrification occurs is much less than the volume of water with active nitrification.

Factors controlling denitrification rates

Factors that influence denitrification in aquatic systems include temperature, the supply of nitrate and organic matter, and oxygen concentration. Further studies are required to make an accurate comparison of the relative importance of these in con-

trolling denitrification rates in streams vs. rivers, lakes, and coastal marine systems.

Only a few studies have considered the effects of temperature on denitrification rates in aquatic systems. The data available generally show increasing rates with increasing temperature. However, because other factors such as nitrification rate and oxygen concentration may also be changing as temperature increases, it is difficult, especially in sediments, to separate the effect of temperature alone.

I have found only one study of the effect of temperature on denitrification in the water column. In Lake Kinneret waters at low oxygen concentrations, denitrification rates increased slightly at temperatures between 15° and 30°C, with a $Q_{10} = \sim 1.35$ (Cavari and Phelps 1977). In sediments, from Duffin Creek, Ontario, the rate of NO_3^- uptake increased by a factor of four when sediment cores collected in winter were warmed from 0° to 20°C (Hill 1983). However, similar laboratory studies using sediment cores from two Danish lakes showed no statistically significant difference in the rate of NO_3^- uptake at temperatures ranging from 5° to 22°C (Andersen 1977). Denitrification rates (acetylene blockage technique) in Lake Okeechobee sediment slurries at saturated NO_3^- concentrations increased as a function of temperature between 14° and 35.5°C, with a $Q_{10} = 2.6$ (Messer and Brezonik 1984).

Denitrification rates (N_2 production from intact cores) in Narragansett Bay increased approximately twofold when the temperature was increased from 3° to 10°C (39 and 69 μmol N m^{-2} h^{-1}, respectively) in sediments collected in March; no further increase was measured when the temperature was increased to 20°C (Seitzinger unpubl. data). The response of denitrification rates to increasing temperatures in that "warming" experiment is similar to the increase in denitrification rates measured at ambient temperatures in March at 3°C (39 μmol N m^{-2} h^{-1}) and at 15° and 23°C in midsummer (100 μmol N m^{-2} h^{-1} at 15° and 23°C) (Seitzinger et al. 1984). In Ochlockonee Bay, denitrification rates were lowest in late winter when temperatures were 17°C and highest in late spring at 21°C, although highest temperatures (25°C) occurred during summer (Seitzinger 1987b). Lower redox conditions and reduced oxygen concentrations

in the sediments at higher summer temperatures in these estuaries may reduce nitrification rates (Jenkins and Kemp 1984) and thus denitrification. In Tama estuary sediments, denitrification rates increased fourfold between 9.3° (December) and 18.0°C (May) (Nishio et al. 1983). An inverse relationship between temperature and denitrification was found in Danish coastal sediments (Sørensen et al. 1979). Highest denitrification rates were measured in January at 3°C and lowest rates in June at 18°C. This was attributed to higher NO_3^- concentrations in the sediments in winter (>100 μM) than in summer (<25 μM).

Numerous laboratory studies using slurries of marine sediments and one study using lake sediments have shown a positive correlation between denitrification rate and nitrate concentration. Those studies usually consist of incubating sediment slurries with various concentrations of nitrate and measuring the rate of decrease in nitrate concentration, N_2 production, or N_2O production in the presence of acetylene. As noted earlier, the response of denitrification is often described by Michaelis-Menten-type kinetic parameters. Half-saturation concentrations for marine sediments using the slurry technique generally range from 27 to 53 μM NO_3^-, with concentrations of 344 μM reported for one study (Table 5). Lake Okeechobee sediments also exhibited a high half-saturation constant, 219 μM. The relationship between denitrification rate and nitrate concentration measured in sediment slurries in the laboratory has been used to estimate in situ sediment denitrification rates. However, as discussed earlier, the calculation is not as straightforward as the results from sediment slurry experiments might suggest. Microenvironmental gradients of nitrate within the sediments are likely to be important in determining ambient denitrification rates (Duff et al. 1984; Jenkins and Kemp 1984), and concentration gradients at these scales are not usually measured. For instance, Jenkins and Kemp (1984) calculated that the distance between sites of nitrification and denitrification was about 80 μm in Patuxent estuary sediments, which suggested that microenvironments within the upper aerated zone of sediments were necessary to maintain such short distances between oxidized and reduced zones.

Table 5. Half-saturation constants (K_m) for denitrification in lake and coastal marine sediments.

Location	Temp (°C)	K_m (µM)	Reference
Lake Okeechobee	35.5	219	Messer and Brezonik 1984
Belgian coast	not reported	50	Billen 1978
Izembek Lagoon	11–15	53	Iizumi et al. 1980
CEPEX sediments	15	31	Koike et al. 1978
Manoku-Ura	8	42	Koike et al. 1978
Tokyo Bay	16	27	Koike et al. 1978
Kysing Fjord	12	344	Oren and Blackburn 1979
San Francisco Bay	20	50	Oremland et al. 1984

Oxygen concentrations can also affect denitrification rates directly and indirectly. Denitrification is generally considered to occur only under low oxygen or anaerobic conditions, although oxygen is required for nitrification. To explain the co-occurrence of these two processes in sediments, investigators have often considered that they are separated vertically within the sediment (Vanderborght et al. 1977; Billen 1978). Alternatively, denitrification may occur within reduced microzones in the aerobic surface layer of sediments (Sørensen et al. 1979; Jenkins and Kemp 1984). These reduced microzones may permit the tight coupling observed between nitrification and denitrification in sediments (Jenkins and Kemp 1984).

Investigations of the effect of oxygen consistently indicate that, in both freshwater and marine systems, an oxygen concentration of ~0.2 mg liter^{-1} or less is required for denitrification in the water or sediment. For example, denitrification occurred in the water of Lake 227 only when the oxygen concentration was <0.2 mg liter^{-1} (Chan and Campbell 1980) and in the water column of the Baltic Sea, when oxygen concentrations were <0.29 mg liter^{-1} (Rönner and Sörensson 1985). In suspensions of coastal marine sediments amended with nitrate (250 µM), no measureable denitrification activity was found at 4.5 kPa O_2 (~2.2 mg O liter^{-1}, assuming 20‰ salinity and 20°C), low rates of denitrification occurred at 0.5 kPa O_2 (~0.25 mg O liter^{-1}), and a sharp increase in activity occurred below 0.25 kPa O_2 (Jørgensen et al. 1984). Further studies investigating the combined effect of nitrate concentration and oxygen concentration on denitrification rates in marine and freshwater sediments are needed.

Differences in oxygen solubility and degree of turbulence between marine and freshwater may affect denitrification rates. Oxygen solubility in freshwater is about 30% greater than in seawater in environmental temperatures, as noted by Capone and Kiene (1988). However, nearshore marine waters are generally more turbulent than lakes, and this may lead to greater oxygen penetration into the sediments and counteract the solubility differences (Capone and Kiene 1988). The effect of oxygen solubility and turbulence on denitrification in aquatic sediments warrants investigation.

A major difference in organic carbon metabolism in freshwater and marine sediments is that in marine sediments sulfate reduction is the predominant pathway of anaerobic metabolism, while in freshwater sediments methanogenesis is predominant (Capone and Kiene 1988). The end-product of sulfate reduction—sulfide—completely inhibits nitrification at concentrations between 0.9 and 40 µM (Yoshida 1967; Srna and Baggeley 1975). Although denitrification is not inhibited by sulfide at concentrations as high as 300 µM (Sørensen et al. 1980), denitrification rates could be indirectly affected by sulfide if nitrification is suppressed. However, the actual importance in marine sediments of sulfide inhibition of nitrification, and indirectly of denitrification, is unclear. For instance, in a eutrophication experiment using the MERL mesocosms, benthic denitrification and nitrification rates (Seitzinger and Nixon 1985) were highest in the sediments with the highest sulfate reduction rates (Sampou unpubl. data).

Denitrification in sediments or anoxic water may also be limited by organic carbon. For example, in the 14–40-cm-deep,

low organic carbon sediments in Great Bay, Long Island, which receive a substantial input of nitrate from groundwater, denitrification was stimulated by the addition of glucose (Slater and Capone 1987). Denitrification rates in the surface sediment were not affected by the addition of organic carbon. Duff et al. (1984) suggested that denitrification did not occur in periphyton communities from a pristine stream even when nitrate was added because of the low dissolved organic carbon content. In the sandy sediments of oligotrophic Lake Hampen, organic carbon released by macrophyte roots was suggested as one of the factors controlling denitrification rates (Christensen and Sørensen 1986). In the hypolimnion of lakes, if organic carbon concentrations are low, organic carbon may be important in regulating denitrification rates (Cavari and Phelps 1977).

Although the organic content may not directly influence denitrification rates in most sediments, it may indirectly affect them since mineralization of the organic matter supplies the ammonia for nitrification. High organic carbon content may stimulate dissimilatory nitrate reduction (Koike and Hattori 1978) which could compete with denitrification for nitrate (Tiedje et al. 1982). In addition, consumption of oxygen during the mineralization of organic matter controls the oxygen distributions in the sediments, which may affect denitrification rates.

Macrophytes, benthic algae, and certain macrofauna have been shown to influence denitrification rates in both freshwater and marine sediments by affecting the oxygen and/or nitrate distribution in the sediments. Denitrification rates in eelgrass (*Zostera marina*) beds were highest where eelgrass biomass was greatest, probably because oxygen was transported through the leaves to the rhizomes and roots in the otherwise anoxic zone of the sediments, thus stimulating nitrification (Iizumi et al. 1980). Similarly, in Lake Hampen denitrification rates were higher in shallow sediments covered with *Littorella* sp. than in nonvegetated areas (Christensen and Sørensen 1986). In the root zone, denitrification was postulated to be controlled by a complex interaction of oxygen transported by the macrophytes which

stimulated nitrification, but may have inhibited denitrification (Christensen and Sørensen 1986). Oxygen produced during photosynthesis by benthic algae may inhibit denitrification. In algal mats covering rocks in a stream, denitrification rates were lower in the light than in the dark (Triska and Oremland 1981). Examination of denitrification and in situ oxygen profiles (using a polarographic microelectrode) in shallow coastal sediments densely covered by benthic microalgae showed a diurnal variation of denitrification rate. Lowest rates of denitrification occurred in the light when oxygen concentrations were highest, and highest denitrification rates occurred in the dark at lower oxygen concentrations (Andersen et al. 1984). Similarly, oxygen produced by benthic microflora in Lake Hampen was postulated to be responsible for the lower denitrification rates in the light than in the dark (Christensen and Sørensen 1986).

Bioturbation by benthic infauna can enhance nitrification and (apparently) denitrification rates in both freshwater and marine sediments. For example, in columns of stream sediment with and without added tubificid worms, the rate of disappearance of $^{15}NO_3^-$ added to the overlying water increased in the presence of the worms (Chatarpaul et al. 1980). In two Danish lakes, the rate of nitrate uptake from the water overlying sediment cores was linearly correlated with chironomid abundance at elevated nitrate concentrations (143–707 μM) (Andersen 1976). Anomalously high nitrate concentrations at 20–30-cm depth in Puget Sound sediments were attributed to nitrification enhancement at those depths due to irrigation by burrowing benthic organisms (Grundmanis and Murray 1977). In the deeper layers of sediments from Kysing Fjord, a secondary peak in denitrification rate was measured in oxidized patches that were probably due to the burrowing activities of macrofauna (Sørensen 1978*b*). In experiment aquaria with Limfjord sediments, nitrification rates in sediments with *Corophium volutator* were higher than those in control sediments (Henriksen et al. 1980). However, the flux of nitrate out of the sediments was similar with and without *C. volutator*. The lack of an increased flux of ni-

trate out of the sediments containing *C. volutator* was attributed to increased denitrification and nitrate reduction rates in those sediments. In a subsequent and similar study, nitrification rates were enhanced in various zones in sediments containing bivalve, crustacea, and annelid infaunal species (Henriksen et al. 1983). Although denitrification rates were reported to be enhanced, no data were presented. Enhanced potential denitrification activity occurred only in the top 0.5 cm of sediments in experimental aquaria with *Nereis japonica* compared to control sediments (Sayama and Kurihara 1983). Potential denitrification activity was measured as the rate of N_2O production in the presence of acetylene in anaerobic sediment slurries highly enriched with a 10 mM NO_3^- solution. All of the above studies indicate that bioturbation enhances nitrification rates. Studies in which denitrification rates are actually measured in the presence and absence of infauna are needed in both freshwater and marine sediments.

Nitrous oxide

N_2O is produced by three microbial processes: denitrification (Knowles 1982); nitrification (Yoshida and Alexander 1970; Goreau et al. 1980); and dissimilatory reduction of nitrate to ammonia (Smith and Zimmerman 1981). N_2O can also be consumed during denitrification (Knowles 1982). Recognition of the role of N_2O in the destruction of stratospheric ozone (Crutzen 1970; Hahn and Crutzen 1982) and in the radiative heat budget of the atmosphere (Wang et al. 1976) has led to many studies of the sources, sinks, and biogeochemical cycles of this trace gas.

Denitrification may be involved in both the production and consumption of N_2O in freshwater and coastal marine systems. In several lakes, undersaturated concentrations of N_2O in oxygen-depleted bottom waters have been attributed to consumption by denitrification (Knowles et al. 1981; Lemon and Lemon 1981; Vincent et al. 1981). Consumption of N_2O by denitrification also appears to be responsible for the low N_2O concentrations in oxygen-depleted coastal marine waters of Saanich Inlet (Cohen 1978) and Chesapeake Bay (Kaplan et al. 1978). No rates of N_2O consumption were reported in any of those studies.

The production of N_2O in aquatic sediments has been demonstrated repeatedly by pore-water profiles of N_2O (Sørensen 1978c; McElroy et al. 1978), as well as by direct N_2O flux measurements from sediment cores (Table 6). Denitrification appears to be the major source of N_2O, as shown in experiments with Danish coastal sediments. N_2O production after the addition of N-Serve, an inhibitor of nitrification, exceeded N_2O production associated with nitrification, except in the narrow range of oxygen concentrations of 0.1–0.2 kPa O_2 (~0.001–0.002 atm O_2) (Jørgensen et al. 1984). The highest total N_2O production rate for the sediments occurred at low oxygen concentrations (<0.5 kPa).

The ratios of net $N_2O : N_2$ production are very small and similar in all lake, river, and coastal marine sediments where measurements are available (Table 6), although the factors that determine the relative amounts of N_2O and N_2 may differ, including oxygen, pH, and H_2S. As the studies below suggest, changes in these factors resulting from anthropogenic inputs may lead to increases in the global production of N_2O. For example, the ratio of $N_2O : N_2$ produced in two lakes in the Pocono Plateau region of Pennsylvania is influenced by pH. In Lake Lacawac, the ratio of $N_2O : N_2$ in benthic fluxes increased by 10-fold (from 0.14×10^{-2} to 1.4×10^{-2}) when the pH of the water overlying the sediment cores was experimentally decreased from 6.6 to 4.4. The $N_2O : N_2$ ratio at pH 4.4 in Lake Lacawac was similar to the ratio in nearby Lake Ernest, where the ambient pH was 4.4. In both lakes the N_2 fluxes were ~50 μmol N m^{-2} h^{-1} (Table 1), including Lake Lacawac when the pH was experimentally decreased to 4.4. The above results are supported by studies with pure cultures of denitrifiers which demonstrate that the amount of N_2O produced relative to N_2 increases under acidic conditions (Knowles 1982).

The effect of oxygen concentration on the $N_2O : N_2$ ratio in coastal marine sediments was shown clearly in a study by Jørgensen

Table 6. Nitrous oxide fluxes from sediments in rivers, streams, and coastal marine systems. Units: μmol N_2O-N m^{-2} h^{-1}.

Location	N_2O flux	$N_2O/N_2 \times 100$	Reference
Rivers			
Potomac			
Gunston Cove	1.1	0.5	Seitzinger 1987*a*
Indian Head	10.0	4.3	Seitzinger 1987*a*
Lakes			
Ernest	0.7	1.2	Seitzinger unpubl. data
Lacawac	0.07	0.14	
Coastal marine			
Tama estuary	1.9	0.32	Nishio et al. 1983
Odawa Bay	<0.1	<0.31	
Limfjorden	−0.4–4		Jensen et al. 1984
Narragansett Bay			
Mid-, lower bay	0.03–0.36	0.1–0.3	Seitzinger et al. 1984
Upper bay	0.06–1.84	0.1–6	
MERL mesocosm	0.56–51	0.2–5.8	Seitzinger and Nixon 1985
Ochlockonee Bay (March 1985)	0.02–0.05	0.02–0.12	Seitzinger 1987*b*
Delaware Bay (July 1985)	0–2.6	0–1.2	Seitzinger unpubl. data

et al. (1984). Using suspensions of Danish coastal sediments, they found that N_2O production by denitrifying bacteria decreased with decreasing oxygen concentrations, although the total rate of denitrification increased. The percentage of N_2O produced dropped rapidly from about 25% at 1 kPa O_2 (~0.987 × 10^{-2} atm) to about 5% at 0.1 kPa. Measured ratios of N_2O : N_2 from marine and freshwater sediments are usually <5%, which suggests that denitrification is occurring at oxygen concentrations <0.1 kPa.

Eutrophication of aquatic systems may result in increased N_2O production rates, as demonstrated in Narragansett Bay. Benthic N_2O fluxes were 1.48 μmol N m^{-2} h^{-1} from the eutrophic upper bay sediments and 0.24 and 0.078 from the relatively unpolluted midbay and lower bay sediments during summer (Seitzinger et al. 1983). Similarly, in a eutrophication experiment using the MERL mesocosms, benthic N_2O fluxes increased dramatically as a function of the rate of nutrient input to the mesocosms (Seitzinger and Nixon 1985). N_2 production and sediment nitrification rates also increased, although by a smaller percentage, as a function of the nutrient input rate. The higher production rates in the more eutrophic sediments may be related to the lower O_2 concentrations in those sediments (Jørgensen et al. 1984). It may also be related to inhibition by H_2S of N_2O reduction to N_2 during denitrification (Sørensen et al. 1980).

Denitrification and benthic mineralization of organic matter

A major part of the primary production in shallow aquatic ecosystems is mineralized in the sediments (Hargrave 1973). The subsequent release of N and P from the sediments is an important source of recycled nutrients for algal production (Nixon 1981; Boynton and Kemp 1985). During the passage of organic matter through the sediments, a large portion of the mineralized nitrogen is lost from the ecosystem via denitrification. This conclusion is based on the percentage of the N flux from aquatic sediments that is N_2 compared to ammonia or nitrite and nitrate. In the two rivers where data are available on benthic ammonia and nitrate fluxes and denitrification rates, ammonia and nitrate fluxes were small or zero while denitrification rates were substantial and accounted for 76–100% of the sediment–water nitrogen flux (Table 4). Ammonia and nitrate fluxes were also small in the four lakes examined. In these lakes, as

in the two rivers discussed above, 74–100% of the sediment–water nitrogen flux was N_2. The data for Lake Michigan are the most extensive as measurements were made over an annual cycle at two locations (Gardner et al. 1987). Denitrification in coastal marine sediments generally accounts for a smaller percentage (between 15 and 60%) of the benthic nitrogen flux compared to lakes and rivers (Table 4), although it can account for 70% more of the benthic nitrogen flux in some coastal marine sediments. The reason for the apparently higher efficiency of removal of mineralized nitrogen via denitrification in freshwater sediments is not known. It may reflect a higher efficiency of nitrification of ammonia produced in freshwater sediments compared to marine sediments. Further studies are required to determine if this pattern holds for lakes and rivers in general.

If the pattern observed for lakes Michigan, Lacawac, and Ernest and the Potomac and Delaware rivers holds generally for freshwater sediments, then little of the nitrogen that is mineralized in freshwater sediments underlying aerobic bottom water is returned to the water column as ammonia or nitrate. In an oligotrophic or mesotrophic lake in which the inorganic nitrogen concentrations in the water are low, the loss of nitrogen via denitrification in the sediments may be a important factor limiting the amount of N available for algal production. In coastal marine systems, denitrification also removes a major portion of the N cycled through the sediments. The fact that phytoplankton in many estuaries appear to be nitrogen-limited may, in part, be due to the loss of nitrogen relative to phosphorus as nutrients are cycled through the sediments (Nixon 1981).

As the above data demonstrate, denitrification removes a large percentage of the organic nitrogen that is mineralized in aquatic sediments. The amount of organic carbon oxidized during denitrification can be estimated by assuming a stoichiometry of 106 g-atoms of organic carbon oxidized per 84.8 moles of nitrate reduced (Richards 1965), i.e. a denitrification rate of 50 μmol N m^{-2} h^{-1} would indicate 62.5 μg-atoms C m^{-2} h^{-1} oxidized. However, few studies have measured denitrification rates concurrently with rates of aerobic and other anaerobic oxidation processes, which makes it difficult to evaluate the relative quantitative importance of denitrification as a pathway of organic carbon oxidation. In Kysing Fjord sediments, denitrification accounted for 22 and 1% of the organic carbon oxidation in winter and summer, respectively, with oxygen accounting for 68 and 96% and sulfate 10 and 4% (winter and summer) (Sørensen et al. 1979). Measurements of oxygen, nitrate, and sulfate reduction along a salinity gradient indicated that denitrification decreased in importance as a pathway of organic carbon oxidation at increasing salinities (Jørgensen and Sørensen 1985). In the brackish region, denitrification accounted for 36% of electron flow, while in the more saline region only 8% of the electron flow was attributed to denitrification.

Denitrification rates vs. nitrogen fixation rates in aquatic ecosystems

Gaseous exchanges of N_2 can be important sources and sinks of fixed nitrogen in aquatic ecosystems. The two microbial processes, denitrification and N_2 fixation, are essentially opposite processes; denitrification removes fixed nitrogen mainly as N_2, and N_2 fixation is a source of fixed nitrogen. The loss of nitrogen via denitrification exceeds the inputs of nitrogen via N_2 fixation in almost all rivers, lakes, and coastal marine ecosystems that have been examined (Fig. 1).

Biological N_2-fixation rates in oligotrophic and mesotrophic lakes are low and rarely account for >1% of either the external inputs of nitrogen to lakes or the nitrogen requirements of the primary producers (Howarth et al. 1988). However, rates in eutrophic lakes are often high. N_2 fixation in eutrophic lakes is often considered to be an important source of nitrogen (Schindler 1977), but the loss of nitrogen from denitrification in many eutrophic lakes (e.g. Wingra, Mendota, and ELA Lake 227 in 1974) exceeds the N_2-fixation inputs by a factor of 2 or more (Fig. 1). An exception to this is ELA 227 during 1975 when increased N_2 fixation is attributed to the low
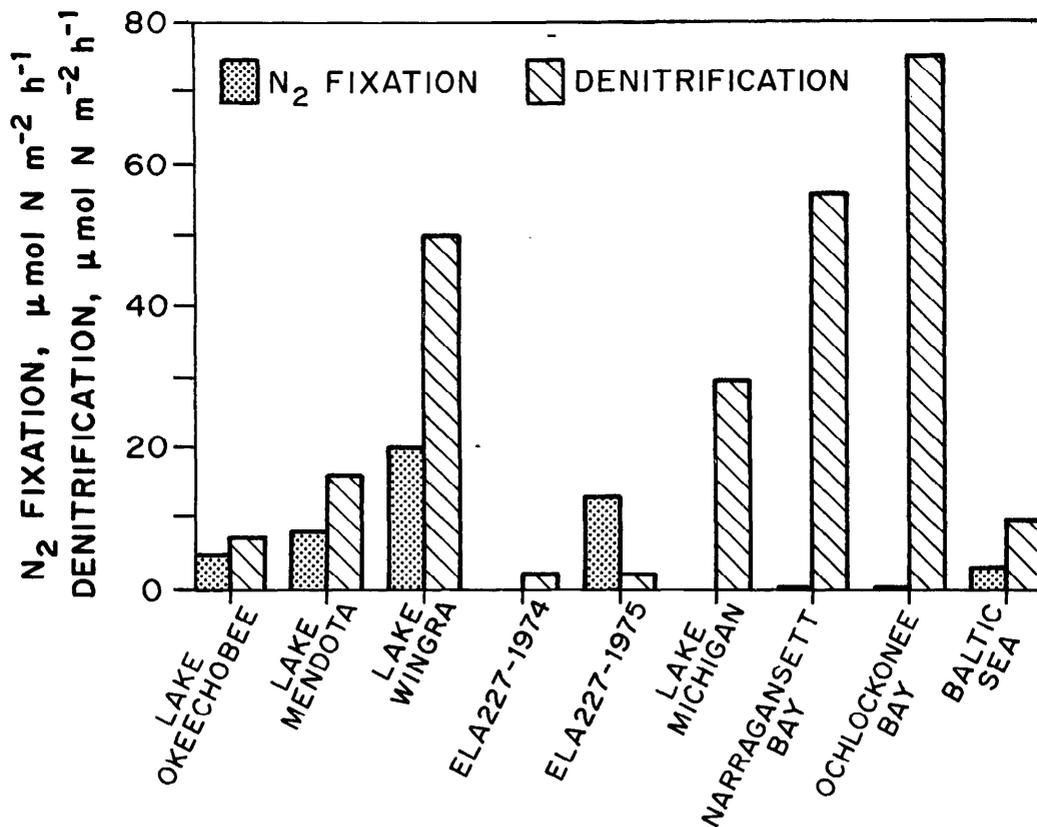
N₂ FIXATION and DENITRIFICATION RATES

Fig. 1. N₂ fixation and denitrification rates reported for lakes and estuaries. Annually integrated rates for lakes Okeechobee (Messer and Brezonik 1983), Mendota (Brezonik and Lee 1968; Keeney et al. 1971), Wingra (Likens and Loucks 1978), and Narragansett Bay (Seitzinger et al. 1984), Ochlockonee Bay (Seitzinger 1987*b*), and the Baltic Sea (Shaffer and Rönner 1984; Larsson et al. 1985). Annual rates of denitrification for the southeastern portion of Lake Michigan (Gardner et al. 1987), N₂ fixation rates taken from the calculation by Howarth et al. (1988) of the data of Mague and Burris (1973). ELA 227 data for summer only (Chan and Campbell 1980; Flett et al. 1980).

N : P ratio of the nutrient loading to the artificially eutrophied lake in 1975 ($\sim 3:1$ by atoms) compared to 1974 ($\sim 6:1$) (Flett et al. 1980). Although in 1975 the average rate of denitrification (~ 15 mg N m^{-2} d^{-1} in summer) (Chan and Campbell 1980) exceeded the average N₂-fixation rate (4.3 mg N m^{-2} d^{-1} in summer), the total input from N₂ fixation exceeded the loss from denitrification because the areal extent of N₂ fixation was greater than that of denitrification which was confined primarily to epilimnetic sediments.

The data in Table 7 show that the removal of nitrogen via denitrification consistently exceeds the input of nitrogen from biological N₂ fixation. However, the data are incomplete, especially in lakes where one or more of the following deficiencies occur in all of the studies: denitrification or nitrogen fixation was only measured in one location or at only one time of year; nitrogen fixation and denitrification rates were not measured during the same year; nitrogen fixation by benthic algae was not measured; denitrification was either measured by techniques that tend to underestimate or overestimate total denitrification rate, or was estimated by mass balance methods.

More complete studies are needed, but

Table 7. The importance of denitrification as a sink for external nitrogen inputs to various aquatic systems. Only systems in which denitrification rates were measured at near-ambient conditions are included. N input, units: μmol N m^{-2} h^{-1}.

Location	N input	N input removed by denitrification (%)	Notes*	Time interval
Lakes				
ELA 227†	3,200	1.4	1	Annual
Kvind søt	1,327	7	2	Annual
Bryrup Langsøt	690	25	2	Annual
Arresø	81	14	3	Annual
Okeechobee	46	9–23	4	Annual
Mendota†	45	36	5	Annual
Rivers				
Potomac	632	35	6	Fall
Delaware	1,452	20	7	Summer
Estuaries				
Tejo estuary	516	45	8	Fall
Delaware Bay	213	46	9	Jul–Sep
Ochlockonee Bay	174	43	10	Annual
Narragansett Bay	112	50	11	Annual
Baltic Sea	25	40	12	Annual
Baltic Sea	25	55	13	Annual
Four League Bay‡		20	14	Annual

* 1. Chan and Campbell 1980; N input calculated based on authors' statement that denitrification rate of 15 mg N m^{-2} h^{-1} equals 1.4% of total fertilizer NO$_3^-$ added to lake (300 kg N). 2. Andersen 1971, 1977; annual denitrification calculated by authors based on rate of uptake of nitrate by sediment cores from overlying water as a function of nitrate concentration in the water, and the nitrate concentration in the lake during the year. 3. Madsen 1979. 4. Messer and Brezonik 1983; range of annual denitrification rates reported by authors based on acetylene blockage assays using sediment slurries and intact cores. 5. Brezonik and Lee 1968; Keeney et al. 1971.

6. Seitzinger 1987a; estimated N input rate in fall from 1980–1983 data of 77,715 lbs N d^{-1} (Thomann et al. 1985) and area of tidal freshwater portion of river including embayments 166 million sq. m (Fitzpatrick pers. comm.). 7. Seitzinger in press; denitrification measured in August 1984 at six locations in the tidal portion of river between Trenton and Marcus Hook. DIN inputs of 83,901 kg N d^{-1} estimated from NH$_4^+$ and NO$_3$ allocations in 1981 for municipal, industrial, and tributary sources to Delaware River between Trenton and Chesapeake–Delaware Canal (Delaware River Basin Commission unpubl. data) and area of river estimated by planimetry.

8. Seitzinger unpubl. data; denitrification measured in November 1983 at eight locations in the bay; N input calculated from unpublished data of Portuguese National Commission for the environment. 9. Seitzinger unpubl. data; denitrification rates measured in September 1984 and July 1985 at eight locations in bay; average rate for bay calculated by applying rate for each location over appropriate areas of the bay. N inputs from Delaware River Basin Commission (unpubl. data). 10. Seitzinger 1987b; denitrification rates measured at five locations throughout the bay over an annual cycle in 1984–1985; N inputs for same time period calculated from N concentrations in Ochlockonee River and river flow, and estimated direct precipitation N inputs. 11. Seitzinger et al. 1984. 12. Shaffer and Rönner 1984; Larsson et al. 1985. 13. Rönner 1985. 14. Smith et al. 1985.

† Minimum denitrification (*see* text).
‡ Only nitrate inputs to estuary measured.

the available data indicate that the losses of nitrogen from denitrification often exceed the inputs of nitrogen from nitrogen fixation in lakes. The net exchange of gaseous nitrogen must be considered when calculating the effect of nitrogen fixation on increasing the N:P ratio of external nutrient input to lakes.

Denitrification as a sink for nitrogen inputs to aquatic ecosystems

The importance of denitrification as a sink for nitrogen input can be evaluated only for the small number of aquatic ecosystems where both have been measured.

Measured denitrification rates and nitrogen input data for only two rivers were found (the Delaware and Potomac). Both receive large amounts of sewage. In the tidal freshwater portion of the Potomac River, the amount of N removed by denitrification during the fall was equivalent to ~35% of the N inputs to that portion of the river (Table 7). Denitrification measured in the tidal, primarily freshwater portion of the Delaware River removed only 20% of the estimated N inputs in summer.

Both denitrification measurements and nitrogen input data were examined for six lakes (Table 7). Denitrification removed an amount of N equivalent to between 1.4 and 36% of the nitrogen inputs in those lakes. The importance of denitrification may be underestimated in five of these lakes because measurements did not include denitrification of nitrate produced in the sediments. Denitrification rates have been inferred by the whole lake mass balance approach for a number of lakes (Table 3).

Again the range is variable: denitrification is estimated to remove an amount of nitrogen equivalent to between 0 and 62% of the external nitrogen inputs. The percentage removed does not appear to be related to either the rate of nitrogen loading or the extent of anoxic bottom water in the lakes. Thorough studies of denitrification and N inputs over annual cycles in lakes are needed to properly assess the magnitude of denitrification as a sink for nitrogen inputs to lakes.

Denitrification removes an amount of N equivalent to between 40 and 50% of the inorganic nitrogen inputs in all but one of the six estuaries for which data are available (Table 7; Fig. 2). The fairly narrow range in the efficiency of removal by denitrification is somewhat surprising considering that these estuaries cover a range of nitrogen input rates, nitrate concentrations in the water column (Delaware Bay > 100 μM in upper bay, Ochlockonee Bay < 10 μM), and extent of intertidal area (40% of the Tejo estuary is intertidal), among other factors. In Delaware Bay and the Tejo estuary, only summer and/or fall denitrification rates are available, and the removal may be somewhat different on an annual basis. The nitrogen input to Four League Bay only includes nitrate. More complete measurements are required to properly evaluate the importance of denitrification as a sink for N in that estuary.

Although the limited number of lakes, rivers, streams, and estuaries in which both denitrification and nitrogen input data are available makes any generalization uncertain, denitrification appears to be important in removing nitrogen inputs in all these systems.

Denitrification not only decreases the amount of fixed N within a system, but it can also decrease the amount of nitrogen transported downstream. In the case of lakes, it may decrease the amount of nitrogen flowing out into streams or seeping into groundwaters. Denitrification in rivers can decrease the amount of N transported to estuaries. In rivers receiving substantial amounts of anthropogenic nutrient input, the removal of nitrogen in the river via denitrification may therefore decrease the degree of eutrophication of coastal marine
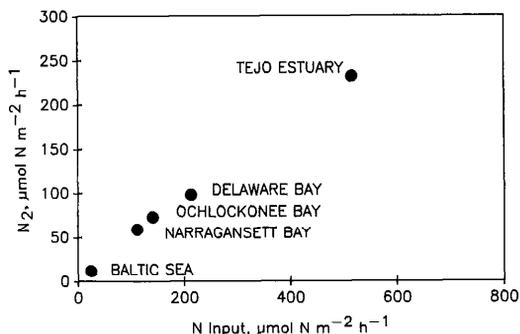

Fig. 2. Denitrification rates vs. external N inputs rates in estuaries. Data explained in Table 7.

waters. Denitrification in estuaries is an important sink for nitrogen in the global marine nitrogen budget. Denitrification decreases by about 40% the amount of continentally derived, riverborne nitrogen transported to the oceans. During glacial periods, when the areal extent of estuaries decreases compared to interglacial times, the percentage of riverborne nitrogen transported to the oceans may increase.

Effect of denitrification on N:P ratios

Denitrification can decrease the N:P ratio in an ecosystem since, during denitrification, nitrogen, but not phosphorus, is removed. For example, in the Baltic Sea the N:P ratio of the predominantly anthropogenic, land-based inputs is 33:1 (atoms), and the N:P ratio in the surface waters is only about 7:1. The low N:P ratio in the surface waters is attributed to denitrification in the water and sediments below the halocline which leads to a very low N:P ratio (2:1) of nutrients returned to the surface waters (Shaffer and Rönner 1984). In Narragansett Bay, the amount of nitrogen removed annually by denitrification is sufficient to decrease the N:P ratio of inorganic nutrient inputs to the bay from 10:1 to 7:1 (Nixon and Pilson 1984). In Ochlockonee Bay, the N:P ratio of inorganic nutrients entering the bay is ~14:1 (Kaul and Froelich 1984; Seitzinger 1987*b*). The amount of nitrogen removed by denitrification is sufficient to decrease the N:P ratio to 8:1 (Seitzinger 1987*b*). In addition to denitrification, the relative amounts of N and P buried and the N_2 fixation inputs must

Table 8. N and P inputs to various lakes and the percent removal of the inputs due to denitrification (DNF), N burial, and P burial. In all lakes, denitrification rates were estimated by authors from mass balance calculations. A negative number indicates a net release from the sediments.

Location	N input	P input	DNF	N burial	DNF + burial	P burial	Reference
	(kg yr⁻¹)		Input (%) removed by				
Norrviken	177,300	10,780	37	25	62	49	Ahlgren 1967
Bryrup Langsø							
1972	32,100	1,490	47	8	56	52	Andersen 1971
1973	30,600	1,170	50	5	55	42	Andersen 1971
Kvind sø							
1972	24,500	2,320	17	16	33	34	Andersen 1971
1973	20,900	1,590	22	0	22	−42	Andersen 1971
Kul sø							
1972	18,800	1,540	18	0	18	−9	Andersen 1971
1973	18,200	2,240	22	5	27	5	Andersen 1971
Salten Langsø							
1972	80,600	11,950	0	20	20	53	Andersen 1971
1973	72,700	9,700	10	19	29	57	Andersen 1971
Halle sø							
1972	27,600	840	54	0	54	−11	Andersen 1971
1973	27,300	700	51	<1	51	6	Andersen 1971
Stigsholm sø							
1972	21,700	1,210	22	0	22	−16	Andersen 1971
1973	22,100	1,880	26	0	26	−11	Andersen 1971
Lake Gardsjon	784	8			42	30	Broberg and Persson 1984
Kinneret							
1968–1969	5,047,000	291,000	62	8	70	86	Serruya 1975
1969–1970	1,524,000	100,800	58	10	68	89	Serruya 1975
Mirror Lake	227	22	3	27	30	76	Likens and Loucks 1978
Lake Wingra	31,357	1,277	26	55	81	94	Likens and Loucks 1978

also be taken into consideration when calculating the expected total N : P ratio in the water column. In general, N and P burial data are lacking for coastal systems.

In a number of lakes, N and P inputs, denitrification, and N and P burial have been estimated (Table 8) and allow insight into the relative importance of these processes in controlling N : P ratios. In most of those lakes, denitrification is a greater sink for N than is burial in the sediments. The percentage of the N inputs removed by denitrification plus burial, and the percentage of the P inputs removed by burial is similar in over half of the lakes. This suggests that, while substantial amounts of nitrogen are denitrified in those lakes, there is no differential loss of nitrogen relative to phosphorus when burial is also considered.

Conclusions and recommendations

Few major differences between denitrification in freshwater and marine ecosystems are apparent based on available data. The range of denitrification rates reported for freshwater and coastal marine ecosystems is similar, although most of the lake denitrification rates fall in the low end of the range for river and coastal marine areas. Nitrification in the sediments is the major source of nitrate for denitrification in most aquatic sediments studied. A large percentage of the organic nitrogen mineralized in rivers, lakes, and coastal marine sediments is denitrified; data available for freshwater sediments indicate that 75–100% of the benthic N efflux is N_2, while in coastal marine sediments generally 20–75% of the ben-

thic N efflux is N_2. In estuaries, denitrification rates generally increase as a linear function of the inorganic nitrogen loading rates with denitrification removing an amount of nitrogen equivalent to 40–50% of the DIN inputs. The relationship between denitrification rates and nitrogen loading rates in streams, rivers, and lakes is not as consistent. The loss of fixed nitrogen via denitrification exceeds the input of fixed nitrogen via N_2 fixation in all the rivers and estuaries, and in all but one lake, where measurements of both processes have been made.

A considerable amount of information is available on denitrification in coastal marine systems, with considerably less information in lakes and streams or rivers. As pointed out throughout this review, there are many areas that warrant further investigation. A few are listed below.

1. In all systems, investigations of the factors controlling denitrification rates are required using ecologically meaningful approaches with single and multiple factor interactions. Factors such as microscale patchiness in sediments of nitrate and oxygen, the effects of bioturbation, and the effect of oxygen concentration in the water column on denitrification rates in the sediments are suggested.

2. How do rates of denitrification change relative to other nitrogen processes (i.e. N input rates, benthic mineralization rates, nitrification rates) from the headwaters to the mouth of streams and rivers?

3. How important is denitrification in removing nitrate inputs from groundwater sources (studies needed in all aquatic systems)?

4. What is the relative magnitude of N_2 fixation and denitrification in lakes? When coupled with phosphorus burial rates, what is the resultant effect on N : P ratios and the algal species composition?

5. Does denitrification remove a larger percentage of the organic nitrogen mineralized in freshwater sediments than in marine sediments? What factors control the relative amount of mineralized nitrogen that is denitrified in freshwater and in marine sediments?

6. How does the total amount of N removed by denitrification in a system change when low oxygen concentrations in the bottom waters are alleviated? What are the management implications?

7. Continued refinements of techniques to measure denitrification are needed. Intercalibration of techniques is also recommended.

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Attachment 2

Valiela, I. and Teal, J.M (1979). The nitrogen budget of a salt marsh ecosystem. *Nature* **280**, 652-656.

The nitrogen budget of a salt marsh ecosystem

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Salt marshes reduce oxidised nitrogenous compounds to ammonium and particulate nitrogen and export these reduced forms to coastal waters. The internal demands exceed the net inputs of nitrogen by rain, groundwater flow and fixation, suggesting very active uptake, conversion, release and recycling of nitrogen within a marsh ecosystem. Nitrogen losses are mainly through tidal exchanges and denitrification, and these two outputs balance the gains. The chemical exchanges among uplands, marshes and coastal water are important in structuring these ecosystems.

NITROGEN is a key nutrient in coastal ecosystems[1]. In salt marshes, nitrogen has a critical role in determining the function and structure of the ecosystem. Salt marsh floras receiving increased amounts of nitrogen show increased rates of primary production[2-5], the abundance of certain species changes and the nitrogen content of the plants increases[6]. Provision of excess nitrogen raises the per cent nitrogen in tissues of marsh grasses from levels typical of C_4 plants to those of C_3 plants[7,8]. This is important because low nitrogen content is one reason why C_4 plants are relatively free from herbivores[9]. The dominant plants (*Spartina alterniflora, S. patens* and *Distichlis spicata*) in salt marshes of the Atlantic coast of the US are C_4 grasses, but where the supply of nitrogen is increased the number of marsh herbivores increases due to the lowered C/N ratios in the plant tissues[6].

Increases in nitrogen supply also lead to increases in the activity and standing crops of decomposer organisms and detritus-feeding invertebrates as the percent nitrogen of the litter helps to regulate decomposers (unpublished results). In general, the biomass of invertebrates in salt marsh plots receiving added nitrogen is larger than in untreated plots. We have also found that increased nitrogen supply changes the morphology of stands of *Spartina alterniflora* from a short to a tall form[10]. Concomitant with this is increased spacing among the taller plants leading to a more open habitat which in turn allows predatory fish and crabs a greater chance of finding prey on the marsh surface[11]. Increases in the supply of nitrogen therefore enhance primary production, decomposer activity, secondary production and changes in the physical structure of a marsh

environment that facilitate predation by fish. Thus the rate of turnover of the entire system to a large extent depends on nitrogen supply.

A second important feature of nitrogen in salt marshes involves the export of nitrogenous materials and marsh-produced organic matter to coastal waters. The proposition that marshes provide food for coastal and estuarine fin and shell fish populations, including economically important species, has been the main argument for conservation of coastal marshes. With the recognition that nitrogen has a limiting role for coastal phytoplankton production[1], it can be appreciated that nitrogen exchange between coastal waters and marshes is significant for both these ecosystems.

We present here the first complete nitrogen budget for a marine ecosystem where each of the various inputs and outputs from a salt marsh has been accounted for. Great Sippewissett Marsh, our study marsh, is located on the western shore of Cape Cod, Massachusetts. Seawater from Buzzards Bay flows into Great Sippewissett Marsh through a single entrance (Sippewissett Creek) and floods the marsh twice daily with a maximum tidal excursion of about 1.6 m. The total area of the marsh is 483,800 m^2 and includes muddy (104,900 m^2) and sandy (61,700 m^2) creek bottoms, algal mats (13,000 m^2), low marsh dominated by short (122,500 m^2) or tall (91,100 m^2) *Spartina alterniflora* and high marsh (89,300 m^2) where the grasses *S. patens* and *D. spicata* are found. The marsh is surrounded by glacial moraine on the south, east and north and by sand dunes to the west. The relief and the ground water table slope generally to the west and towards the marsh and bay.

Inputs and outputs

Nitrogen enters Great Sippewissett Marsh through precipitation and flow of groundwater, the latter flowing into the marsh through numerous small underground springs. Other sources of nitrogen are fixation of atmospheric nitrogen by blue-green algae and bacteria in the sediments. Tidal flushing brings in and removes dissolved and suspended nitrogen from the marsh. The second major loss of nitrogen is through denitrification. Other minor fluxes include volatilisation of ammonia, removal of shellfish, loss to sediments and deposition of faeces by roosting birds.

Groundwater flow. Groundwater was sampled over 2 yr at seven small underground springs that flowed from the upland into the marsh[12]. The amounts of ammonium, nitrite and organic nitrogen were determined using an autoanalyser. The total flow of groundwater into the marsh lowered the salinity of the ebbing tidal water by about 2‰. This lowered salinity was used to calculate the volume of freshwater contributed by groundwater. Salinities were collected routinely during entire tidal cycles through the year. With these estimates of concentrations and volumes, the amount of each nutrient entering the marsh at different times of the year was calculated (Fig. 1). Nitrate was the major inorganic nitrogenous constituent of groundwater (averaging over 50 μmol l^{-1}), with substantial amounts of dissolved organic nitrogen (DON) (Table 1). There was a small reduction of nitrate flow during the autumn (Fig. 1). The amount

of ammonium increased in summer, presumably due to the increased decay of litter prompted by higher temperatures at that time of year. The amounts of nitrite involved were minimal and were not included in Fig. 1. The total annual input of nitrogen to Great Sippewissett Marsh by groundwater was 6,120 kg yr⁻¹.

Precipitation. The amount of precipitation and the concentration of ammonium, nitrate, nitrite, DON and particulate nitrogen were measured in rain gauge collections at various sites over 2 yr (ref. 12). Nutrient inputs for the four seasons was calculated by multiplying the concentrations of nitrogenous nutrients by the amount of rain for each rainfall. The nutrient concentrations were typical of semirural areas[13-15] and there was little seasonal change. Nitrate and DON were the principal forms of nitrogen delivered by rain (Fig. 2 and Table 1). The concentration of all nutrients was higher in the groundwater than in precipitation[12] primarily due to evapotranspiration by terrestrial vegetation. There is little evidence of any increase in nitrogenous concentration in groundwater due to leaching from septic tanks upstream. The volume of rain that fell on the marsh was much less than that which reached the marsh as groundwater, so only 380 kg yr⁻¹ of nitrogen reached the marsh through rainfall (Table 1).
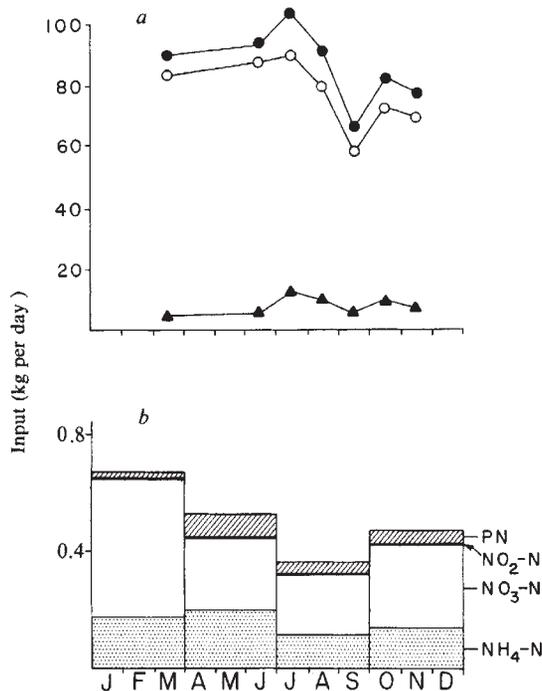
Fig. 1 Amounts of nitrogenous nutrients brought in by groundwater flow (a) and precipitation (b) into Great Sippewissett Marsh throughout the year. Values for precipitation are averaged for 3 months periods. Even though rain fell in episodic fashion, results are averaged on a per day basis over an entire 3-month period for ease of comparison with the other inputs and outputs. ●, Dissolved organic nitrogen; ○, NO₃—N; ▲, NH₄—N.

Nitrogen fixation by bacteria. Fixation of nitrogen by bacteria in the marsh is due to free-living bacteria in sediments lacking vegetation and bacteria closely associated with roots of marsh plants[16]. Rates of bacterial fixation in each of the marsh habitats were measured by incubating cores of sediment for 24 h in a gas mixture of 86% nitrogen, 10% acetylene, 4% oxygen. We then reflushed the headspace above the core with gas of a similar composition and measured ethylene production 24 h later by gas chromatography[17].

Low marsh was the major site of bacterial fixation due to the higher rates (100–500 ng N cm⁻² h⁻¹ during the warm part of

the year) and larger area involved (Fig. 2). High marsh had lower rates (50–150 ng N cm⁻² h⁻¹) and smaller area. Fixation peaked in the vegetated habitats during spring and early autumn, when roots were actively growing or senescence was taking place. Perhaps root exudates are most abundant at those times. Fixation rates in creek bottoms were considerably lower (maximum of 80 and 20 ng N cm⁻² h⁻¹ for muddy and sandy creeks) than in those recorded in sediments supporting plants and peak fixation occurred in midsummer (Fig. 2). Nitrogen fixation totalled 2,980 kg yr⁻¹.

Table 1 Nitrogen budget for Great Sippewissett Marsh (kg yr⁻¹)

Processes	Input		Output	Net exchange
Precipitation		380		380
NO₃—N	110			
NO₂—N	0.4			
NH₄—N	70			
DON	190			
Particulate N	15			
Groundwater flow		6,120		6,120
NO₃—N	2,920			
NO₂—N	30			
NH₄—N	460			
DON	2,710			
N₂ fixation		3,280		3,280
Algal	297			
Rhizosphere bacteria	2,595			
Non-rhizosphere bacteria	384			
Tidal water exchange		26,200	31,600	−5,350
NO₃—N	390		1,210	
NO₂—N	150		170	
NH₄—N	2,620		3,540	
DON	16,300		18,500	
Particulate N	6,740		8,200	
Denitrification			4,120 (2,820)	−6,940
Sedimentation			1,295	−1,295
Volatilisation of NH₃			17	−17
Deposition of bird faeces		9		9
Shellfish harvest			9	−9
Totals		35,990	39,860	−3,870

Totals for each process are offset to the right. Losses from the marsh to Buzzards Bay are shown as negative numbers in the net exchange column. The amount of nitrogen added to the denitrification value is not equal to the amount of nitrogen fixed because net denitrification was not active in the blue green algal mats. Some of the values in this table and in the text may not agree with those of refs. 12, 16, 18, 20. The entries here were computed using corrected areas for the habitats and supersede earlier estimates.

Nitrogen fixation by algae. Algal fixation was measured for each habitat by an adaptation of the acetylene reduction technique[18]. Although pannes and algal mats had the highest fixation rates (up to 500–600 ng N cm⁻² h⁻¹), the area of these habitats was so small that their contribution of N to the total marsh was small. Low marsh and creek bottoms showed lower rates[18] but their larger areas resulted in a greater contribution to the nitrogen economy of the marsh. The total nitrogen introduced into the marsh by algal fixation was 297 kg yr⁻¹, an order of magnitude smaller than that contributed by bacterial fixation.

Tidal exchange. The flow of seawater and nitrogenous nutrients through the mouth of Sippewissett Creek were measured during entire tidal cycles about monthly throughout the year. Water movement in and out of the marsh was measured with mechanical flow meters throughout each tidal cycle[12]. Tidal water was sampled hourly during each cycle and the concentrations of dissolved and particulate nutrients were determined. With these data and knowledge of the cross-sectional area and tidal height of Sippewissett Creek, water flux and nitrogenous nutrients entering and leaving the marsh during each hour of a tidal cycle were calculated. We summed over the tidal cycle to obtain net exchange (Fig. 3a).

Ammonium was the principal form of dissolved inorganic nitrogen (DIN), averaging about 1 μmol NH₄—N l⁻¹ in winter and spring and 2–6 μmol NH₄—N l⁻¹ in summer and autumn. For the first half of the year there were only trivial exchanges of

ammonium between the marsh and Buzzards Bay (Fig. 3a). From June to July there was a substantial import of ammonium into the marsh. This took place while the vegetation was actively growing and setting seed. The growth rate of S. alterniflora in Great Sippewissett Marsh is such that this species by itself consumes more than 39 kg per day of NH_4—N. During the same time of year about 9 kg per day of NO^-_3 and NH^+_4 nitrogen are brought into the marsh daily by groundwater (Fig. 3b). The tidal import of DIN into the marsh (Fig. 3b) was 8 kg per day, making a daily total of 17 kg DIN per day, a rate lower than the 39 kg per day used by plants during their period of active growth.

Towards the end of August there was a marked export of ammonium into Buzzards Bay (Fig. 3a), related to the maturity and senescence of marsh vegetation. The peak export of ammonium from the marsh was after the plants had matured and began flowering (Fig. 4); mature and senescent plants are most subject to leaching[19]. In a salt marsh with twice daily submergence in a good electrolyte, leaching should be important. Measurements of leaching of NH_4—N from marsh grasses show that such losses peak in August and that the standing crop of marsh grasses can release about 7 kg N per day through leaching (unpublished results).

In late summer the DIN conveyed into the marsh by groundwater (about 8 kg N per day), is no longer intercepted by the marsh ecosystem. Adding this amount of groundwater DIN to the 7 kg N per day leached from plants, a value (15 kg N per day) that is near the observed export of DIN (roughly 12 kg N per day) from the marsh to the Bay in late August and September is obtained (Fig. 3b). Perhaps additional ammonium is released by anaerobic respiration of plant exudates. In August the sediments become markedly more anoxic (B. Howes, personal communication). We are investigating the relationship between root activity and redox condition but as yet are unable to estimate the contribution of this mechanism to tidal export of DIN.

Nitrite is found in low concentration (up to 0.2 μg—at NO_2—N1^{-1}) in marsh water and it is difficult to know if the fluctuations seen in Fig. 3 (top) are of significance. Nitrate concentrations were higher (averaging up to 1 μmol 1^{-1}), with net exports in autumn and winter (Fig. 3a).

Figure 3b shows the seasonal changes of total dissolved inorganic nitrogen. The marsh intercepts most of the groundwater DIN (mostly nitrate) during winter and spring. This may be due to the conversion of nitrate dissolved in the water into nitrogen gas by denitrifying organisms, described later. During midsummer the marsh imports DIN, as the grasses take up ammonium during maximum growth. With the onset of senescence, plant leaching and decay lead to export of DIN. The coming of winter slows all these processes. Throughout most of the year the marsh intercepts DIN (principally nitrate) that otherwise would flow into deeper waters.

Denitrification. In low and high marsh—habitats where plant roots consolidate sediments—rates of denitrification were measured using a bell jar method. Darkened glass bell jars were pushed about 20 cm into the sediment and the headspace was flushed with helium. Argon was injected into the headspace as an internal standard. After a 4-h incubation, samples of the gas in the headspace were removed and the ratio of nitrogen-to-argon was measured by gas chromatography.

The denitrification rates of the wet flocculent sediment of creek bottoms and pannes were assayed using a gas-partitioning technique. Samples of the sediment were placed in serum vials and flushed with helium. After incubation for 24 h the gas in the sediment was stripped onto the headspace by shaking. The gases were then sampled and analysed by gas chromatography[20].

The rates of denitrification were measured monthly at each of the habitats (Fig. 2). Due to the higher rates (maximum of 5 mg N m^{-2} h^{-1}) and large area of creek bottoms[20], this habitat was the site of over 50% of the net denitrification for the marsh. The low marsh had peak denitrification rates of 2–3 mg N m^{-2} h^{-1}, while the high marsh rates were only ~1 mg N m^{-2} h^{-1}. The rates in the pannes reached over

5 mg N m^{-2} h^{-1} but their small surface area did not make a large contribution to losses of N from the marsh as a whole. In the algal mats the high fixation rates were matched by high rates of denitrification[20]. In all habitats there was a strong seasonal pattern associated with the annual temperature cycle.

Both techniques used to measure denitrification yield results that represent the difference between true denitrification rates and simultaneous nitrogen fixation within the sediment. In Table 1 we added the measured amounts of nitrogen fixed to the
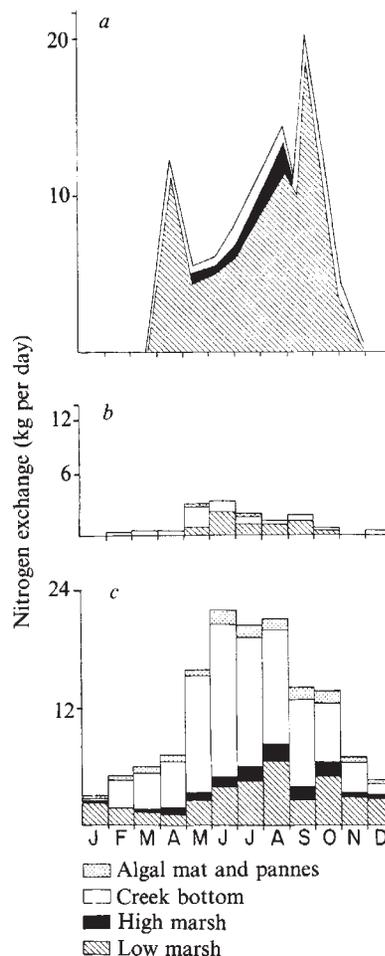
Fig. 2 Nitrogen exchanged by microbial processes in Great Sippewissett Marsh. Each major habitat is shown separately. Values are the products of rates × areas of each of the habitats. a, Bacterial N_2 fixation; b, algal N_2 fixation; c, denitrification.

amount of nitrogen lost by denitrification to obtain the total amount denitrified. This rate of total denitrification shows that low marsh and creek bottoms are the habitats where denitrifiers are most active and contributed the most to losses of N by denitrification (Fig. 2). The other habitats are of minor importance and the whole marsh exports about 6,940 kg N yr^{-1} as nitrogen gas.

Nitrate is not exported during the spring and summer. This is the time of year when rates of denitrification are highest, suggesting that denitrifiers are active enough to substantially alter the chemistry of tidal water.

Losses to sediments. The rate of accretion of sediments was obtained from measurements made for marshes near Great Sippewissett[21,22]. For well-established vegetated zones, we used a value of 0.15 cm yr^{-1} as the rate of sediment accumulation. In sediments of Great Sippewissett Marsh there is an average of

1.4% N and the specific gravity of 0.2 g dry weight cm^{-3}. We estimate that in the area stabilised by the growth of vegetation (302,900 m^2), 1295 kg N yr^{-1} is lost to the sediments. Although the loss of nitrogen to sediments is substantial relative to other inputs and outputs (Table 1), the amount of nitrogen sedimented is only about 1% of the estimated 116,800 kg N stored in the top 15 cm of marsh sediments. The bulk of the roots of marsh plants are found above a depth of 15 cm (ref. 23) and therefore any nitrogen found above 15 cm could be potentially recirculated by plant uptake. We are concerned here with losses that occur when nitrogen is left below a depth of 15–20 cm as the marsh peat accumulates.

Minor sources and losses. Our value for the amount of nitrogen lost from Great Sippewissett Marsh through volatilisation of NH$_3$ is based on rates measured in North Carolina[24]. At the range of pH values (5–8) measured in our marsh sediments, volatilisation would be small (Table 1).

Flocks of gulls use the marsh as a roost. As they feed largely elsewhere, we consider their defaecation to be an input of nitrogen. We calculated this small input (Table 1) by noting the average number of birds in the marsh, the average defaecations per unit time and the weight and per cent of nitrogen in the faeces.

There is active shellfishing in Great Sippewissett Marsh. The removal of nitrogen by this pathway was estimated by using the records kept by the shellfish warden and the per cent nitrogen in the shellfish meats. This is also a small figure (Table 1).

The balance sheets

Table 1 summarises annual nitrogen transport. The sum of the inputs is remarkably close to the sum of the outputs (only about 11% difference). This balance would be required for long-term persistence of an ecosystem in its present state.

Although adequate replication and estimates of variation for each process are available[12,16,18,20], as only one marsh was studied the variability for each entry in Table 1 cannot be easily estimated. There is the further problem that even very small errors in, for example, rates of flow of tidal water may be more important than larger errors in measurements of nutrient concentrations. This is inherent in the arithmetic used in making the estimates of Table 1. From our experience and by pooling estimates of variation from the data available[12,16,18,20], we believe that the coefficient of variation of each entry of Table 1 would be about 20% of the mean. Thus, the small 11% variation between inputs and outputs of Table 1 is fortuitous. This agreement suggests that major parts of the nitrogen budget have been accounted for.

Does the marsh have much flexibility to adjust to increased eutrophication of ground and tidal waters? Of the total nitrogen budget of Great Sippewissett Marsh, 91% of the inputs and 83% of the outputs were driven by physical forces which might indicate little room for adjustment. However, if the refractory (see below) dissolved organic nitrogen is not considered, less than 50% of the exchanges are purely physical and the possibility of considerable biological adjustment may be indicated. As dissolved ammonium increases, nitrogen fixation is sharply curtailed[25]. Fixation amounts to 9 or 20% of the input, again depending on whether or not the DON is considered an inert fraction of the nitrogen budget. In either case this biological feedback system can make only a relatively small change in the system's ability to handle increased nitrogen inputs. Perhaps increased rates of denitrification with increased supply of nitrogen can also serve as a feedback mechanism for adjusting nitrogen flow through the marsh. Our evidence is not conclusive[26], although others have shown increased denitrification after nitrate enrichment[27]. To be effective, increases would have to be large and our rates are already among the higher values reported[20]. Salt marsh sediments show very large rates of sulphate reduction[28]. As nitrate is energetically more favourable as an electron acceptor than sulphate, large

increases in nitrate reduction are possible if new supplies of nitrate are made available.

Internal mechanisms, nevertheless, are unlikely to regulate the amount of nitrogen entering salt marshes. Eutrophication of entering fresh and tidal waters will probably result in an increase of the nitrogen pool within the marsh, especially in sediments and vegetation. This will to some extent enhance primary production in salt marshes[2-5]. Human contamination affects principally ground and tidal water, the two largest processes providing nitrogen to the marsh. Of the two, contamination of groundwater may be more important as flow is one way into the
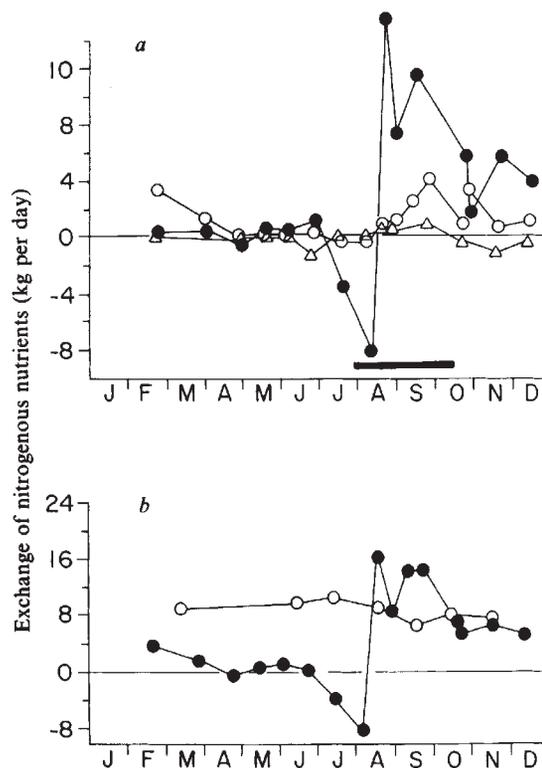
Fig. 3 Exchanges of nitrogenous nutrients between Great Sippewissett Marsh and Buzzards Bay through the year. In *a*, points are net exchanges for a given tidal cycle and recalculated in units of kg per day. ●, NH$_4$—N; ○, NO$_3$—N; △, NO$_2$—N. The solid bar shows the flowering season for *Spartina alterniflora*. *b*, Compares net tidal exchanges of dissolved inorganic nitrogen (●) with the dissolved inorganic nitrogen furnished by groundwater (○) flowing into the marsh. Values in both graphs above the 0 in the *y* intercept are net exports from Great Sippewissett Marsh, while negative values show an import into the marsh.

marsh and, at least in North America, disposal of sewerage wastes is conducted primarily by leaching wastewater through cesspools and septic tanks into the soil. This may result in enhanced concentrations of nutrients, particularly nitrate, in groundwater throughout the coastal zone.

A better view of the action of a salt marsh ecosystem as a transformer of chemical forms of nitrogen can be obtained by rearranging the entries of the nitrogen budget. The annual exchanges of the major forms of nitrogen for Great Sippewissett Marsh are shown in Table 2. Sixty-four percent of the nitrogen entering the marsh as nitrate is intercepted by the marsh. The oxidised nitrogen imported into the marsh is reduced to various forms within the marsh. Ultimately most of the entering nitrogen is exported by tidal water in the form of particulate, ammonium and molecular nitrogen.

These exports are substantial. Export of dissolved inorganic nitrogen from the area of marshland around Buzzards Bay at a rate comparable to that found in Great Sippewissett Marsh in

Table 2 Annual exchanges of major forms of nitrogen for Great Sippewissett Marsh ($kg\,yr^{-1}$)

Form of N	Input	Output	Net	% of input
NO_3-N	3,420	1,220	2,200	64
NH_4-N	3,150	3,550	−400	−13
DON	19,200	18,500	700	4
Particulate N	6,750	8,200	−1,460	−22
N_2	3,280	6,940	−3,660	−112
Totals	35,800	38,410	−2,610	−7

The exchanges of nitrite and the nitrogen lost to the sediments are not included.

August could entirely replace the nitrate content of Buzzards Bay in 100 days[12]. The amount of particulate organic matter exported yearly from the marsh is equivalent to about 40% of the net annual above ground production of the marsh[12]. As salt marshes are among the most productive ecosystems on a per unit area basis, these amounts exported are quantitatively significant and must be important to detritus feeders in coastal waters.

As annual denitrification rates exceed fixation, there are large losses of nitrogen from the marsh as N_2 gas. This is the second major pathway of nitrogen export.

The large amounts of dissolved organic nitrogen (Tables 1, 2) are mostly unaffected by passage through the marsh. This agrees with other results that suggest that the bulk of DON is refractory to microbial attack[29], although DON is the principal form of nitrogen in terms of total amounts.

Approximately 6,940 kg of nitrate nitrogen are required to supply the marsh denitrification annually. This is a larger amount than is available through the net import of nitrate ($2,200\,kg\,yr^{-1}$, Table 2). This means that some nitrification of ammonium must take place within the sediments.

The amount of ammonium entering the marsh is composed of three sources; (1) net exchange by tides, groundwater and rain ($1,450\,kg\,N\,yr^{-1}$); (2) the small amount of mineralisation of DON ($700\,kg\,N\,yr^{-1}$); and (3) the amount fixed by micro-organisms ($3,280\,kg\,N\,yr^{-1}$). The total of these three sources is $5,430\,kg\,N\,yr^{-1}$. After allowing $1,460\,kg\,N\,yr^{-1}$ for export of particulates and $1,295\,kg\,N\,yr^{-1}$ for losses to sediments, only $2,675\,kg\,N\,yr^{-1}$ are available for use within the marsh. Yet we estimate that growth of marsh plants must use about $8,800\,kg\,N\,yr^{-1}$. Thus, it is clear that very active uptake, release, conversion and recycling of nitrogen must take place within the marsh.

If the salt marsh were not present, Buzzards Bay would receive the nitrate from the groundwater and the phytoplankton would be the principal part of the Bay's ecosystem that would be affected. The marsh intercepts much of this nitrate and exports primarily particulate nitrogen to the sea. This export principally affects the animals that feed on particles, a very different component of the coastal food web. These interactions between marsh and bay maintain the structure of benthic and fish communities in the bay. Similarly, the flow of groundwater structures the marsh biota by providing dissolved inorganic nitrogen for plants and denitrifiers.

These results demonstrate that there are significant interactions among units of landscape, in our case nutrient exchanges

among upland, marsh and coastal waters. In view of their importance it is obvious that these interactions among ecosystems have not received enough attention. Many aspects of the structure of an ecosystem, such as what plants, animals or microbes are present, and how many of each kind, are strongly affected by nutrients delivered from outside the particular ecosystem.

Conclusions

Our findings show that there are significant exchanges of nutrients between upland, marsh and coastal waters, and these interactions affect the structure and composition of these systems.

Groundwater often imports more N into salt marshes than does precipitation. Contamination of coastal zone groundwater by sewage is probably important to the marsh nitrogen economy. However, tidal water causes a net export of all nutrients studied, especially ammonium and particulate nitrogen. Quantitatively, tidal exchange is the most important nitrogen transport mechanism. Ammonium export seems to be linked to the activity of marsh grasses, and uptake and leaching are important mechanisms. Nitrogen fixation by bacteria is larger than that by blue-green algae and most bacterial fixation is associated with the roots of marsh grasses.

Dissolved organic nitrogen is the major form of N in the water but little of this is available to decomposers. Despite this rates of denitrification are high. Denitrification may be responsible for the interception by the marsh of the nitrate transported by the flow of groundwater. Annual denitrification for the entire marsh exceeds nitrogen fixed, causing a net export of N_2 to the atmosphere.

Loss of nitrogen to deeper sediments is significant, but this is small in proportion to the nitrogen pool found in the surface sediments where plant roots are found.

The amount of dissolved inorganic nitrogen required to satisfy growth of higher plants and supply the nitrate for denitrification is larger than imports of DIN, which suggests that nitrification, nitrogen fixation and active recycling of N are all important within the marsh. Nitrogen export by the marsh is also important for coastal waters and the transformation of oxidised imports to reduced exports is significant to the coastal ecosystems that receive marsh exports.

Altogether, the imports and exports of nitrogen are in balance. This, along with other evidence, suggests that an old salt marsh such as that studied is in a long-term steady state. Young marshes may be traps for sediment and particulate nutrients suspended in tidal water, but this becomes less marked as the marsh develops, with mature marshes exporting particulates.

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Attachment 3

U.S. Census Bureau, American Fact Finder. Income in the Past 12 Months from the 2009-2013 American Community Survey for the Town of Marion.

S1901	INCOME IN THE PAST 12 MONTHS (IN 2013 INFLATION-ADJUSTED DOLLARS)
	2009-2013 American Community Survey 5-Year Estimates

Supporting documentation on code lists, subject definitions, data accuracy, and statistical testing can be found on the American Community Survey website in the Data and Documentation section.

Sample size and data quality measures (including coverage rates, allocation rates, and response rates) can be found on the American Community Survey website in the Methodology section.

Although the American Community Survey (ACS) produces population, demographic and housing unit estimates, it is the Census Bureau's Population Estimates Program that produces and disseminates the official estimates of the population for the nation, states, counties, cities and towns and estimates of housing units for states and counties.

Subject	Marion town, Plymouth County, Massachusetts				
	Households		Families		Married-couple families
	Estimate	Margin of Error	Estimate	Margin of Error	Estimate
Total	1,911	+/-130	1,386	+/-104	1,201
Less than $10,000	2.8%	+/-2.3	1.6%	+/-1.4	0.6%
$10,000 to $14,999	4.5%	+/-2.4	0.9%	+/-1.3	0.0%
$15,000 to $24,999	8.4%	+/-4.3	6.0%	+/-4.9	4.5%
$25,000 to $34,999	4.2%	+/-2.8	1.6%	+/-1.6	1.5%
$35,000 to $49,999	6.9%	+/-3.2	7.9%	+/-4.2	4.6%
$50,000 to $74,999	17.2%	+/-5.8	17.0%	+/-7.5	18.3%
$75,000 to $99,999	17.6%	+/-5.4	18.0%	+/-6.5	18.7%
$100,000 to $149,999	18.7%	+/-5.6	22.7%	+/-6.9	25.1%
$150,000 to $199,999	4.3%	+/-2.2	6.0%	+/-3.1	6.9%
$200,000 or more	15.3%	+/-5.2	18.4%	+/-6.6	19.8%
Median income (dollars)	80,456	+/-7,949	92,258	+/-21,486	107,898
Mean income (dollars)	112,586	+/-15,115	127,702	+/-17,263	N
PERCENT IMPUTED					
Household income in the past 12 months	34.9%	(X)	(X)	(X)	(X)
Family income in the past 12 months	(X)	(X)	38.5%	(X)	(X)
Nonfamily income in the past 12 months	(X)	(X)	(X)	(X)	(X)

Subject	Marion town, Plymouth County, Massachusetts		
	Married-couple families	Nonfamily households	
	Margin of Error	Estimate	Margin of Error
Total	+/-108	525	+/-152
Less than $10,000	+/-1.0	6.1%	+/-7.6
$10,000 to $14,999	+/-2.9	14.1%	+/-8.1
$15,000 to $24,999	+/-4.5	14.7%	+/-8.3
$25,000 to $34,999	+/-1.7	11.0%	+/-9.2
$35,000 to $49,999	+/-3.4	4.2%	+/-4.6
$50,000 to $74,999	+/-8.6	20.8%	+/-12.6
$75,000 to $99,999	+/-7.4	16.8%	+/-13.6
$100,000 to $149,999	+/-7.3	5.1%	+/-6.0
$150,000 to $199,999	+/-3.6	0.0%	+/-6.4
$200,000 or more	+/-7.3	7.2%	+/-6.4
Median income (dollars)	+/-24,388	44,911	+/-27,223
Mean income (dollars)	N	70,966	+/-25,824
PERCENT IMPUTED			
Household income in the past 12 months	(X)	(X)	(X)
Family income in the past 12 months	(X)	(X)	(X)
Nonfamily income in the past 12 months	(X)	25.5%	(X)

Data are based on a sample and are subject to sampling variability. The degree of uncertainty for an estimate arising from sampling variability is represented through the use of a margin of error. The value shown here is the 90 percent margin of error. The margin of error can be interpreted roughly as providing a 90 percent probability that the interval defined by the estimate minus the margin of error and the estimate plus the margin of error (the lower and upper confidence bounds) contains the true value. In addition to sampling variability, the ACS estimates are subject to nonsampling error (for a discussion of nonsampling variability, see Accuracy of the Data). The effect of nonsampling error is not represented in these tables.

While the 2009-2013 American Community Survey (ACS) data generally reflect the February 2013 Office of Management and Budget (OMB) definitions of metropolitan and micropolitan statistical areas; in certain instances the names, codes, and boundaries of the principal cities shown in ACS tables may differ from the OMB definitions due to differences in the effective dates of the geographic entities.

Estimates of urban and rural population, housing units, and characteristics reflect boundaries of urban areas defined based on Census 2010 data. As a result, data for urban and rural areas from the ACS do not necessarily reflect the results of ongoing urbanization.

Source: U.S. Census Bureau, 2009-2013 5-Year American Community Survey

Explanation of Symbols:

 1. An '**' entry in the margin of error column indicates that either no sample observations or too few sample observations were available to compute a standard error and thus the margin of error. A statistical test is not appropriate.
 2. An '-' entry in the estimate column indicates that either no sample observations or too few sample observations were available to compute an estimate, or a ratio of medians cannot be calculated because one or both of the median estimates falls in the lowest interval or upper interval of an open-ended distribution.
 3. An '-' following a median estimate means the median falls in the lowest interval of an open-ended distribution.
 4. An '+' following a median estimate means the median falls in the upper interval of an open-ended distribution.
 5. An '***' entry in the margin of error column indicates that the median falls in the lowest interval or upper interval of an open-ended distribution. A statistical test is not appropriate.
 6. An '*****' entry in the margin of error column indicates that the estimate is controlled. A statistical test for sampling variability is not appropriate.
 7. An 'N' entry in the estimate and margin of error columns indicates that data for this geographic area cannot be displayed because the number of sample cases is too small.
 8. An '(X)' means that the estimate is not applicable or not available.