LETTERS

Reversal of the net dinitrogen gas flux in coastal marine sediments

R. W. Fulweiler[1], S. W. Nixon[1], B. A. Buckley[1] & S. L. Granger[1]

The flux of nitrogen from land and atmosphere to estuaries and the coastal ocean has increased substantially in recent decades. The observed increase in nitrogen loading is caused by population growth, urbanization, expanding water and sewer infrastructure, fossil fuel combustion and synthetic fertilizer consumption[1,2]. Most of the nitrogen is removed by denitrification in the sediments of estuaries and the continental shelf, leading to a reduction in both cultural eutrophication and nitrogen pollution of the open ocean[3,4]. Nitrogen fixation, however, is thought to be a negligible process in sub-tidal heterotrophic marine systems[5]. Here we report sediment core data from Narragansett Bay, USA, which demonstrate that heterotrophic marine sediments can switch from being a net sink to being a net source of nitrogen. Mesocosm and core incubation experiments, together with a historic data set of mean annual chlorophyll production[6,7], support the idea that a climate-induced decrease in primary production has led to a decrease in organic matter deposition to the benthos and the observed reversal of the net sediment nitrogen flux. Our results suggest that some estuaries may no longer remove nitrogen from the water column. Instead, nitrogen could be exported to the continental shelf and the open ocean and could shift the effect of anthropogenic nitrogen loading beyond the immediate coastal zone.

Climate change has altered the phenology or seasonal sequencing of events in a variety of ecosystems[8,9]. In the marine environment, the timing of phytoplankton production is particularly important because it affects both pelagic and benthic ecology[10,11]. For shallow systems, organic matter deposited to the benthos determines fauna abundance as well as rates of biogeochemical cycling[12,13]. In turn, the fluxes of nutrients off the sediment surface can influence water-column phytoplankton production, especially when pelagic nutrient concentrations are low. The impact of climate change on this benthic–pelagic coupling has previously been unknown.

Long-term warming[14,15] has resulted in the loss of the winter–spring diatom bloom in most years and a decrease in mean annual chlorophyll production between the 1970s and 1990s in Narragansett Bay, Rhode Island (41.3° N, 071.1° W)[6]. This decrease has continued through 2005 and there is also a marked decrease in summer chlorophyll concentrations (Fig. 1). The exact mechanism behind this decline is not known, but it is believed to be the result of increased year-round grazing[15] or an increase in cloudy days, which negatively affects the onset of the winter–spring bloom[16] (Supplementary Discussion). Nitrogen inputs have remained constant for the last twenty-five years[17]. Combining the chlorophyll observations with associated Secchi disk data (a proxy for light penetration depth)[18] in a simple empirical model that relates carbon fixation by marine phytoplankton to chlorophyll, optical depth, and surface irradiance[19] suggests that primary production in the mid-bay may have declined by 40% in the last thirty years. Recent measurements in the bay have also shown a marked decrease in sediment denitrification, sediment oxygen consumption, and in the fluxes of ammonium and phosphate from sediments to the overlying water compared with extensive measurements made in the 1970s and 1980s[7].

As in most estuaries, denitrification has traditionally been a dominant nitrogen process in the sediments, removing between 15–25% of the nitrogen load from land and atmosphere to the bay[3,20]. But during the summer of 2006, using the N_2/Ar technique[21,22], we observed a dramatic change in the net sediment N_2 flux by mid-bay sediments (Fig. 2). High rates of N-fixation were also found in triplicate cores collected in July and August at three other stations throughout the bay (Table 1). The N-fixation was seasonal, and by mid-September the sediments were once again dominated by denitrification. These N-fixation rates are remarkable for two reasons. They represent some of the highest rates measured for a marine system[23], and this is the first demonstration that heterotrophic marine sediments can switch from being a net sink to being a major net source of nitrogen. We hypothesized that the decrease in primary production led to a decrease in organic matter deposition to the benthos and the observed reversal of the net sediment N_2 flux (Supplementary Discussion). We tested this hypothesis using core incubations and nine large (4 m^2) benthic mesocosms containing sediment and associated fauna from mid-bay.

The mesocosm experiment was designed to quantify the response of the benthos to different levels of organic deposition. Estimates of the proportion of phytoplankton blooms that fall to the bottom in coastal waters vary widely, with larger values for winter–spring blooms and

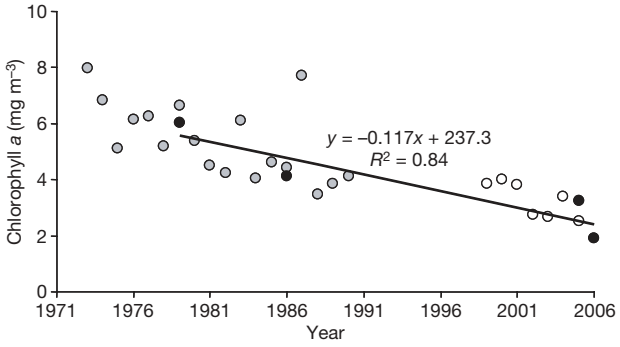

Figure 1 | Multi-decadal mean annual water column chlorophyll *a* concentrations. Mean annual water column (surface and bottom) chlorophyll *a* concentrations in mid-bay over the past three decades. Grey circles are from ref. 6; open and closed circles from the Graduate School of Oceanography plankton monitoring programme (http://www.gso.uri.edu/phytoplankton). Black circles are the mean summer (June, July, August) chlorophyll *a* concentrations shown for the years when N_2 fluxes were measured at the mid-bay station (41° 35.3′, 071° 22.3′). Regression is for the mean summer chlorophyll *a* values.

[1]Graduate School of Oceanography, University of Rhode Island, Narragansett, Rhode Island 02882-1197, USA.

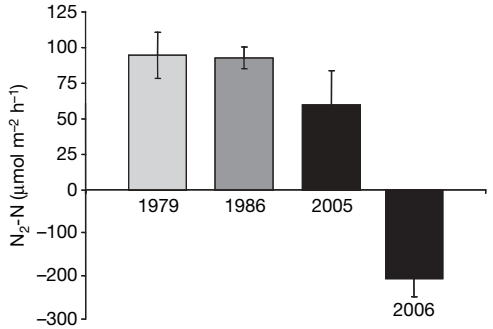

Figure 2 | Mean summer (17–23 °C) N_2 fluxes in mid-bay. Positive rates indicate denitrification, negative rates indicate N-fixation. 1979 data are from ref. 29 (mean ± s.d., $n = 2$), 1985/86 data are from ref. 30 (mean ± s.d., $n = 7$), 2005 data are from ref. 7 (mean ± s.d., $n = 6$), and 2006 data are from this study (mean ± s.d., $n = 6$). Historical denitrification rates are significantly (one-way ANOVA; $P = 0.002$) higher than those measured in 2005/06 (see the Methods).

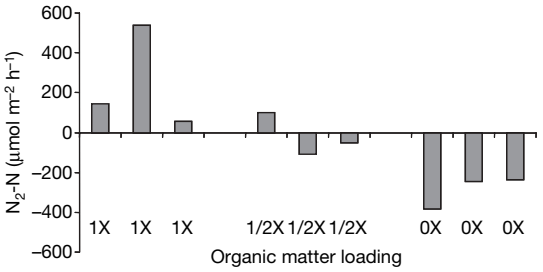

Figure 3 | Net N_2 fluxes measured across a gradient of organic matter loading in mesocosms at the Graduate School of Oceanography. Sediments were collected from the mid-bay historic site. Sediments labelled 1X received a total of 50 g C m^{-2}, 1/2X systems received 25 g C m^{-2}, and 0X systems received no organic matter. Positive rates indicate net denitrification, negative rates indicate net N-fixation.

lower values for summer blooms[24]. We assumed a deposition of 25% of four equal blooms based on a June through September carbon fixation of 200 g C m^{-2} measured in mid-bay water during the early 1970s[25]. Each mesocosm was randomly assigned to one of three levels of organic addition in the form of commercial spray-dried marine phytoplankton with a C:N ratio of 10.5. Beginning in June, three mesocosms received no organic enrichment (0X), three received the spray-dried plankton in four monthly aliquots of 6.25 g C m^{-2} (1/2X) each, and three received 12.5 g C m^{-2} (1X) each. Our intention was that the 50 g C m^{-2} (1X) total summer deposition might approximate the field condition as it was during the 1970s.

We measured the net sediment N_2 flux at the end of the experiment in September 2006. Organic matter loading had a progressive effect on net sediment N_2 fluxes, with denitrification proceeding in the most heavily enriched treatments, both denitrification and nitrogen fixation occurring at relatively low rates in the medium-enrichment treatments, and only nitrogen fixation taking place in the non-enrichment treatments (Fig. 3). Field-collected sediment cores were confirmed to exhibit net nitrogen fixation in early September 2006 at rates similar to the non-enriched mesocosms. After this determination, we added spray-dried plankton to the cores to replicate the 1X enrichment. Two weeks later these cores had also switched from N-fixation (-250 μmol N_2-N m^{-2} h^{-1}) to denitrification (530 μmol N_2-N m^{-2} h^{-1}). Thus, only a brief exposure to organic matter was needed to reverse the net sediment N_2 flux.

N-fixation associated with cyanobacteria mats and seagrass beds can be an important nitrogen source in shallow, oligotrophic, tropical systems[5]. In contrast, benthic N-fixation has historically accounted for a very small percentage of the total system nitrogen in deeper temperate estuaries and bays (that is 0.1% in Narragansett Bay[26] to 4% in Rhode River, Chesapeake Bay[5]). Preliminary analysis of the sediments at the mid-bay station found no cyanobacteria pigments, suggesting that heterotrophic bacteria are responsible for the N-fixation. This is consistent with the fact that all N-fixation incubations were made in complete darkness and the mesocosms were maintained for four months in the dark before the N-fixation measurements.

Table 1 | N-fixation during the summer of 2006 in Narragansett Bay

Site	Latitude Longitude	Temperature (°C)	N_2-N (±s.d.) (μmol m^{-2} h^{-1})
1	41° 46.7′	18	−25 (±3.5)
	071° 22.8′	22	−245 (±9.7)
2	41° 43.1′	18	−30 (±5.2)
	071° 21.6′	22	Not measured
3	41° 43.2′	18	−135 (±19.4)
	071° 18.5′	22	−650 (±200.4)
4*	41° 35.3′	18	−75 (±13.2)
	071° 22.3′	22	−276 (±90.4)

* Historic station discussed in the text.

Simultaneous nitrogen fixation and denitrification have been observed in shallow, subtropical, estuarine sediments[27]. The N_2/Ar technique measures net N_2 production or uptake, so it is impossible for us to know whether this was the case for these temperate sediments. However, it is possible to determine whether the net N_2 flux is positive (that is removing nitrogen through denitrification) or negative (that is adding nitrogen through N-fixation) to the bay over the annual cycle. If we apply the N-fixation rates observed at the mid-bay site in the summer of 2006 to the entire bay, we estimate that N-fixation introduced $(1.4–2.2) \times 10^6$ moles of nitrogen per day. This is roughly 1.5 times greater than nitrogen inputs over the summer from the land and atmosphere combined[17]. This is a conservative estimate as N-fixation rates measured at the other three stations were higher (Table 1). Because the N-fixation is seasonal, the major nitrogen addition occurred from June through August and amounted to $(125–200) \times 10^6$ moles of nitrogen during this period. If we apply the mean annual denitrification rate observed during 2005 (40 μmol N_2-N m^{-2} h^{-1}) to the whole bay over the remaining nine months of the year, denitrification could remove about 86×10^6 moles of N. Thus, over the complete annual cycle in 2006 the sediments were adding a net $(40–110) \times 10^6$ moles of nitrogen to the bay. This is equal to 20–60% of the annual nitrogen input from direct sewage discharge.

During summer, dissolved inorganic nitrogen concentrations throughout Narragansett Bay proper are very low and strongly limit primary production[28] (Supplementary Fig. 1). In the past, this summer nitrogen limitation was eased by the remineralization of nitrogen deposited on the bottom during the winter–spring bloom. Presumably, the dissolved inorganic nitrogen that used to be taken up by the winter–spring bloom is now flushed offshore. But the climate-induced oligotrophication of the bay has also now made the sediments a new source of reactive nitrogen. It is too soon to know whether this source will become large enough to replace the dissolved inorganic nitrogen being lost offshore during winter. The conversion of the sediments from a significant sink for N_2 over the annual cycle to a large nitrogen source illustrates the complex interplay of climate and biology as they influence biogeochemical cycling in the coastal environment. With these observed changes, a new model for nitrogen dynamics in coastal systems is emerging. Some estuaries may no longer provide the nitrogen retention and removal services they once did. Instead, nitrogen may be exported from the estuary to the continental shelf and, perhaps, to the open ocean, thus shifting the effect of anthropogenic nitrogen loading beyond the immediate coastal zone.

METHODS

Field sampling. Three replicate sediment cores (of 10 cm inner diameter and 30.5 cm long) were collected from the mid-bay site using SCUBA divers. Sediment cores (of 10 cm inner diameter, 30.5 cm long) from the three other sites were collected using a 5 m pull corer onboard the R/V *Zostera*. The cores

were stored at *in situ* temperatures and taken back to the laboratory at the Graduate School of Oceanography of the University of Rhode Island, where they were placed in a water bath and maintained at field temperatures in a dark environmental chamber. The cores were left uncapped with air bubbling gently through the overlying water for about 8–12 h. Before the net N_2 flux incubation the water overlying each core was carefully replaced with filtered (1 μm) lower Narragansett Bay (~32 practical salinity units) water. The cores were then sealed with a gas-tight lid (with no air headspace) and replicate water samples for N_2/Ar analysis were collected at five points over the course of an incubation and preserved with $HgCl_2$. Incubations at 17–23 °C lasted between 6 and 24 h.

Mesocosm experiment. We used a large box corer to collect sediments in May 2006 to a depth of about 30 cm from near the mid-bay station where the long-term chlorophyll measurements are made and close to the historical site of mid-bay benthic fluxes (41° 35.3′, 071° 22.3′). We took great care not to disturb the sediments and to preserve their vertical structure. The sediments were maintained in the dark in nine 4 m^2 mesocosms with 65 cm of overlying water, which was slowly mixed and exchanged at 10% each day with water from the lower West Passage of the bay. The sediments in the field are at 7 m (mean low water, mlw) with a 1.1 m tidal range, receive less than 1–2% of surface illumination, and are completely heterotrophic. In September sediment cores (of 10 cm inner diameter, 30.5 cm long) were collected from each of the mesocosms with a pull corer and transported to the environmental chamber for the N2/Ar incubation as described above.

Laboratory analysis. Dissolved gas samples were analysed with a quadrupole membrane inlet mass spectrometer (MIMS, Bay Instruments) that only requires a small sample size (<10 ml) and no sample preparation. The instrument provides rapid throughput (~20–30 samples per hour) and a precision of ±0.03% (ref. 22). The N_2/Ar ratio is actually a measure of net N_2 production or consumption (gross denitrification minus gross nitrogen fixation). For the N_2/Ar method, the change in N_2 concentration was determined from the change in the measured N_2/Ar multiplied by the Ar concentration at air saturation. N_2 production for each of the triplicate cores was then determined from a five-point linear regression (Supplementary Fig. 2). Rates were then corrected for the volume of water overlying the core and area of the core. With assistance from M. Hayn at Cornell University and L. Cole from the University of Virginia, we confirmed that N-fixation was occurring at three of the sites, using the more traditional acetylene technique.

Statistical analysis. Differences between the historical and most recently collected sediment oxygen demand, sediment net N_2 fluxes, and the fluxes of ammonium and dissolved inorganic phosphate across the sediment water interface and their relationship with temperature were examined with the statistical analysis program SAS, using a two-way analysis of variance (ANOVA) and least-significant-difference multiple comparison test. All of the fluxes in this study were significantly ($P < 0.01$) lower than those measured previously. Using ANOVA we also tested to see if the historical summer (17–23 °C) N_2 fluxes in mid-bay were different from each other. The historical summer rates are significantly ($P = 0.002$) different from those measured in 2005 and in 2006.

Cyanobacteria analysis. Six surficial (0–2 cm) sediment samples were sent to the laboratory of H. Paerl at the University of North Carolina for cyanobacteria extraction[31]. No cyanobacteria indicator pigments could be found.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions R.W.F. was responsible for sediment collection as well as sample and data analysis for net N_2 measurements. R.W.F. and S.W.N. co-wrote this manuscript. B.A.B. was responsible for statistical analysis. B.A.B and S.L.G. supervised the large-scale mesocosm experiment.

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