

Effects of Non-indigenous Oysters on Ecosystem Processes Vary with Abundance and Context

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ABSTRACT

Invasive species can alter the structure and functioning of ecosystems and affect the quality of the services they provide. Effects on biodiversity are well documented, but less is known about their impacts on ecosystem functioning and how these change as their populations increase. Invasive oysters, *Crassostrea gigas*, were added at increasing abundances to two different estuarine habitats (mussel-beds and mud-flats) and biotic and functional changes were assessed after 15 months. Sediment–water fluxes and benthic turnover rates of ammonium were greatest at medium cover of *C. gigas* in both habitats, but for silicate they increased with increasing cover of oysters in mud-flats but, by contrast, decreased at the highest cover of oysters in mussel-beds. Community respiration increased with the greatest cover of oysters in both habitats. Biodiversity was increased by *C. gigas* in mud-flats, but decreased with

the greatest cover of oysters in mussel-beds. Assemblage structure differed between habitats and among different covers of oysters and 33% of the total variation in assemblage structure correlated with the variation in nutrient fluxes and concentrations, suggesting possible direct or indirect effects of *C. gigas* on the relationship between biodiversity and ecosystem functioning. *C. gigas* can alter biodiversity and benthic turnover rates of important limiting nutrients, and may therefore affect provision of ecosystem services. Predicting the effects of invasive species requires knowledge of how their influence varies with abundance and among receiving environments.

Key words: ecosystem functioning; invasive species; biodiversity; ecosystem engineer; *Crassostrea gigas*.

INTRODUCTION

Non-indigenous invasive species can alter native biodiversity (Bax and others 2003) and ecosystem functioning (Ehrenfeld 2010) thereby affecting the provision of ecosystem services (Pejchar and

Mooney 2009). Furthermore, damage caused by invasive species profoundly affects economies worldwide (Pimentel and others 2001), thus necessitating effective management and mitigation strategies. Evaluating the costs of invasive species to society is difficult, but important if decisions involving tradeoffs between investing in the control of invasive species or control of other pressures on ecosystems are to be made (Oreska and Aldridge 2011).

Although the effects of invasive species on biodiversity have been widely documented, quantitative

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evaluation of their effects on ecosystem functioning are still rare (Ehrenfeld 2010). Invasive species can alter processes important for the functioning of ecosystems, such as biogeochemical cycling rates (Kurten and others 2008), nutrient availability (Gomez-Aparico and Canham 2008), productivity (Sousa and others 2008), rates of decomposition (van der Putten and others 2007), and community respiration (Martin and others 2007). These changes to ecosystem processes can be driven directly by the invader itself, or indirectly via changes to local biodiversity induced by the invader. Alterations to biodiversity may alter the magnitude and stability of ecosystem processes, and therefore may exacerbate effects on ecosystem functioning (Gamfeldt and Hillebrand 2008). Similarly, alterations to ecosystem processes may generate feedbacks that further exacerbate changes to biodiversity (Duke and Mooney 2004). These mechanisms may operate simultaneously, to varying degrees, depending on the species and/or process being affected. Hence, it is important for researchers to measure and understand a number of processes, such as community respiration, photosynthesis, and biogeochemical cycling (Gamfeldt and others 2008), because the impact of an invasive species may affect some processes but not others.

The nature and magnitude of the effects of invasive species may also depend upon the characteristics of the receiving environment (Queirós and others 2011) and on the abundance of the invading organism (Thomsen and others 2011). Abundance will vary spatially and temporally at different stages of establishment in and subsequent invasion of non-native habitats. Understanding of the variation in impacts of invasive species at different abundances and within different habitats has been identified as a key gap in empirical knowledge of biological invasions (Sousa and others 2009; Thomsen and others 2011), particularly concerning effects on ecosystem functioning (Ehrenfeld 2010).

Invasive “ecosystem engineers” (Jones and others 1994) cause some of the most significant changes to the physical and/or chemical properties of native ecosystems (Cuddington and Hastings 2004). The Pacific oyster, *Crassostrea gigas*, is an ecosystem engineer that can construct very dense reefs (in some cases over 2,000 individuals per m²; Markert and others 2010). Invasive populations of *C. gigas* have been found throughout much of the world and although their effects on biodiversity have been extensively documented (see Troost 2010 for review), their effects on ecosystem functioning remain largely unexplored. In addition

to inducing changes through the physical structure of their shells (Lenihan 1999; Lejart and Hily 2011), *C. gigas* filter-feeds, removing suspended phytoplankton and inorganic particulates from the water column and depositing feces or pseudofeces onto the benthos. Such biodeposits may lead to carbon and nitrogen accumulation (Haven and Morales-Alamo 1966) and anoxia in sediments (Kaspar and others 1985). High mineralization rates of biodeposits can increase nutrient turnover and increase the release of ammonium and silicate into the water column (Christensen and others 2003). These biogeochemical changes may also affect infaunal biodiversity and community structure (Tenore 1982). The extent to which ecosystem processes and services are altered, however, is also likely to depend on the cover of *C. gigas* that is established and on the nature of the habitat being invaded. For example, the effects of *C. gigas* becoming established in habitats already dominated by biogenic reef forming organisms, such as mussel-beds, would be expected to differ from those caused by its establishment in habitats without biogenic reefs, such as mud-flats.

In the current study, the cover of *C. gigas* was experimentally manipulated in the field to determine (1) how *C. gigas* affects ecosystem functioning in invaded habitats, (2) how the effects of *C. gigas* differ between mussel-bed and mud-flat habitats, (3) how these effects change with increasing cover of *C. gigas*, and finally (4) the extent to which the effects on biodiversity and ecosystem functioning co-vary, using data on biodiversity collected simultaneously but reported elsewhere (Green and Crowe unpublished data).

MATERIALS AND METHODS

Study Site

This experiment was done at Lough Swilly (Ballylin Point, County Donegal, Ireland: 55°2′36.12″, –7°33′36.09″) on lower intertidal mussel-bed and mud-flat habitats. Mussel-bed habitats were characterized by dense populations of the blue mussel, *Mytilus edulis*, on sedimentary substrata whereas mud-flat habitats were large patches of mud substrate (~5 to 20 m²) interspersed between mussel-beds and not dominated by mussels or any other habitat-forming species. Sediment within mussel-beds was a mixture of fine sand and silt with large shell fragments throughout. Sediment within mud-flats was a mixture of very fine sand, silt, and clay.

Experimental Design

The experiment was set up during late April 2009, with two orthogonal factors; habitat (2 levels, mussel-beds and mud-flats) and cover (4 levels: zero (0%), low (5%), medium (50%), or high (100%) cover of *C. gigas*). These four levels of cover were applied to 50 × 50 cm plots in each habitat and equated to final densities of 0, 16 ± 0.5, 120 ± 8, and 240 ± 12 individuals per m² and biomasses of 0, 26.06 ± 2.13, 390.88 ± 31.95, and 781.77 ± 63.90 g per m² ash-free dry weight. All oysters used in this experiment were found in situ and were not moved from other locations. Oysters were rinsed with seawater and cleaned of any epibionts prior to deployment and were then simply inserted upright into the mud-flat and mussel-bed habitats to simulate how they are typically found in natural populations. Four replicate plots were randomly allocated to each treatment and were sampled after 15 months (July 2010).

Organic Matter (OM) Content and C/N Ratios

Sediment samples were collected from three depths (0–2, 2–4, and 4–6 cm) using a mini-corer adapted from a 60 ml syringe. At each plot, five sediment samples were taken from each depth and pooled together prior to analysis to account for spatial heterogeneity within plots. OM content was determined by loss on ignition (Eleftheriou and McIntyre 2005) and expressed as a percentage of ash-free dry weight. In addition, total organic carbon (TOC %) and nitrogen (TON %) were determined on oven dried (80°C) samples of the surface layer (0–2 cm). Prior to analysis 50 mg of pulverized sediment was weighed into silver capsules and pre-treated with HCl to remove carbonates (Hedges and Stern 1984). Total C and N were determined using the Dumas principle of complete and instantaneous oxidation of the sediment by sample combustion at 950°C with oxygen injection on an Elementar vario EL cube.

Pore-water Nutrients

Pore-water samples were collected using purpose-built Rhizon™ (PES, Polyester Sulphone membranes) in situ profilers modified from the design of Seeberg-Elverfeldt and others (2005). These consisted of perspex sheets into which grooves were cut at certain intervals to allow attachment of Rhizon™ soil moisture samplers on a vertical sequence. Rhizons™ were 10-cm long with 0.1 μm pore size placed

onto the profilers at 0 (sediment–water interface), 1, 2, 3, 4, 5, 8, and 10 cm depths. Profilers were carefully inserted into the sediment and left for 24 h prior to sampling to reduce the effects of sediment disturbance upon insertion. Overlying surface water (~2–4 mm above the sediment–water interface) was collected from plots using separate Rhizons™. This method allows pore-water profiles to be sampled directly into sterile vacuum tubes with minimum disturbance of the benthic structure to a vertical resolution of 1 cm (Seeberg-Elverfeldt and others 2005; Rocha and others 2009). All water samples were stored in the collecting vacuum tubes and were subsequently analyzed for ammonium (NH₄⁺), nitrate (NO₃), nitrite (NO₂), and silicate (Si(OH)₄⁻) using a Lachat Quick-Chem 8000 flow injection autoanalyzer with Lachat methods 31-107-06-1-B (ammonia), 31-107-04-1-A (nitrite and nitrate), and 31-114-27-1-A (silicate). Concentrations of NO₂ and NO₃ were always below the detection limit and were omitted from further analyses. Pore-water nutrient concentrations were then corrected for porosity and were standardized to dry bulk density (Eleftheriou and McIntyre 2005).

Diffusive fluxes of NH₄⁺ and Si(OH)₄ across the sediment–water interface were calculated from vertical pore-water concentration gradients according to Fick's first law of diffusion. Concentration gradients were determined from the sediment pore-water data using simple linear regression (Moore and others 1991) and were used to estimate the diffusive nutrient flux. Calculations were based on the linear portion of the nutrient profiles at the sediment–water interface (from surface water to 1 cm depth). Diffusion coefficients (cm² h⁻¹) of 0.064 for ammonium and 0.024 for silicate were used in the calculations after being corrected for porosity and tortuosity (Schulz and Zabel 2000).

NH₄⁺ and Si(OH)₄ inventories were calculated by depth integration of the pore-water concentrations corrected for porosity down to 10 cm depth. Residence times for NH₄⁺ and Si(OH)₄ were then calculated as the ratio of inventory to diffusive flux rates.

Community Respiration

Gas samples were obtained using the closed chamber technique (Hutchinson and Mosier 1981) from 8 L chambers fitted with airtight rubber septums. Cuvettes were painted black to eliminate light and measure community respiration. Samples were taken at hourly intervals for 3 h from time

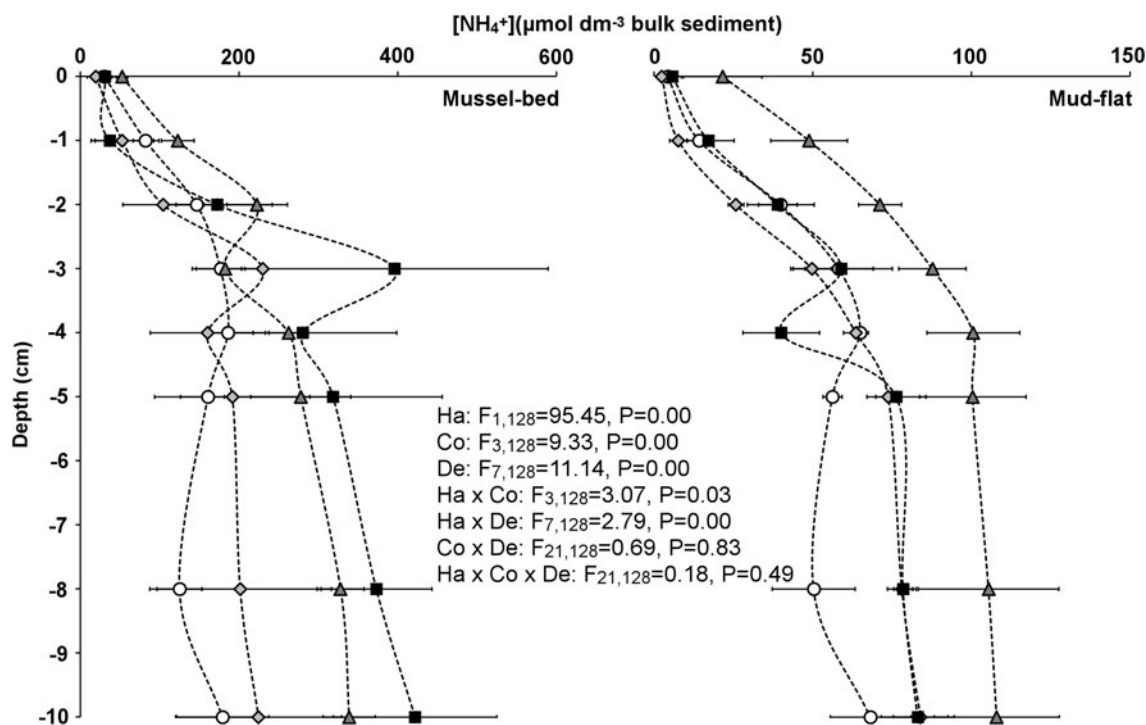


Figure 1. Pore-water ammonium. Concentration of NH_4^+ in pore-water from the sediment–water interface (0 cm) down to 10 cm depth in experimental plots with 0 (white circle), 5 (light gray diamond), 50 (dark gray triangle), or 100 (black square) % cover of oysters in mussel-beds or mud-flats. ANOVA F ratios and P values are included, mean \pm SE.

zero with 60 ml syringes closed with a 3-way stopcock. The air column overlying the sediment inside the chambers was mixed by gently pumping the syringe three times before each sample was taken. Temperature was measured inside the cuvettes using a thermometer and a concurrent estimation of atmospheric pressure was made using online data from Met Éireann. Samples were analyzed for CO_2 , N_2O , and CH_4 using a gas chromatographer (Shimadzu GC-2024) with an automated injection system (Loftfield and others 1997). The flux rates were calculated using the ideal gas law and linear regression using the chamber temperature and average air pressure during cover period (Hutchinson and Mosier 1981).

Biological Assemblages

Epiflora and fauna were counted or recorded as percentage cover of a 25×25 cm quadrat randomly placed onto plots. An additional sediment core with a 10 cm cross-section was then taken down to 10 cm depth in each plot. The cored samples were processed to extract epifauna and infauna according to protocols described in detail elsewhere (Green and Crowe unpublished data).

Statistical Analyses

Differences in OM %, diffusive fluxes of NH_4^+ and $Si(OH)_4$ and measured gas fluxes of CO_2 , CH_4 , and N_2O were evaluated using 2-factor analyses of variance (ANOVA) based on the design described in the “Experimental Design” section. The factors “habitat” and “cover” were both treated as fixed. Homogeneity of univariate variance was tested using Cochran’s C test. When this was significant data were fixed by square-root transformation to avoid the probability of inflated Type I error rates (Cochran 1947; Underwood 1997). When significant differences were detected by ANOVA ($P < 0.05$), Student-Newman-Keuls (SNK) tests were done to identify patterns of difference. All calculations were done using Win-GMAV (Underwood and Chapman 1998).

A distance-based linear model (DISTLM) procedure was used to perform a permutational test of the null hypothesis of no relationship between biological assemblages and functional variables (Legendre and Anderson 1999) calculated on Bray–Curtis similarity measures (Bray and Curtis 1957) and analyzed using the PRIMER package (PRIMER-e 2009). Functional variables included in the

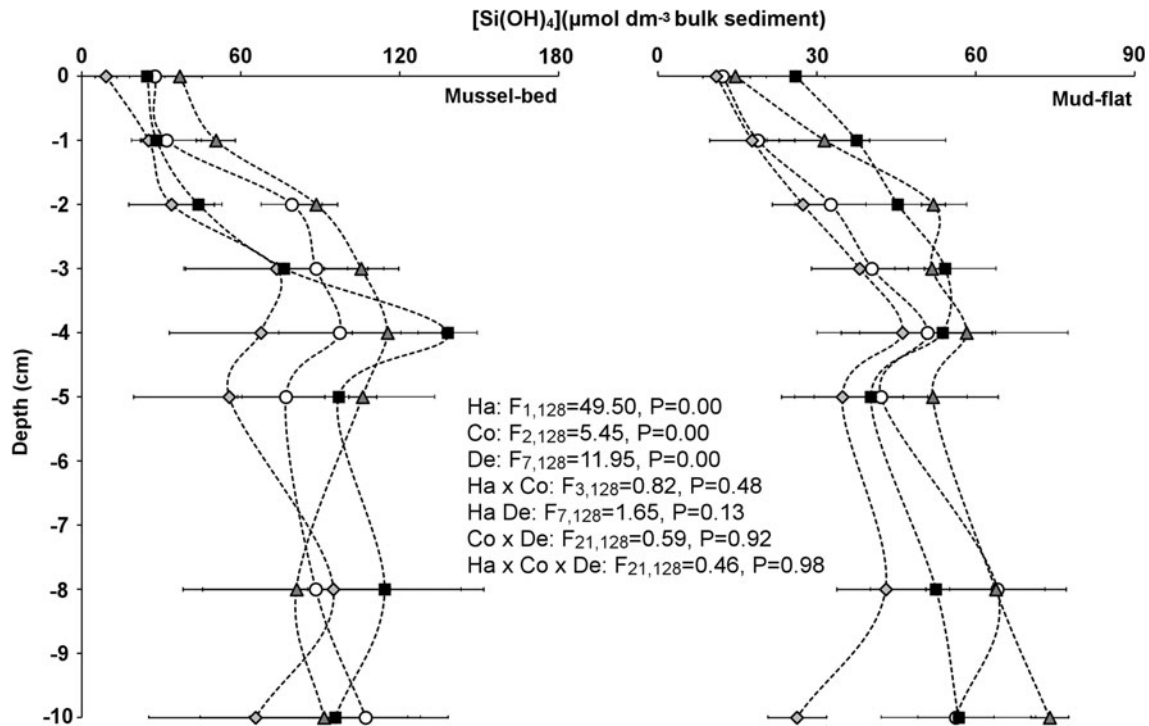


Figure 2. Pore-water silicate. Concentration of Si(OH)_4 in pore-water from the sediment–water interface (0 cm) down to 10 cm depth in experimental plots with 0 (white circle), 5 (light gray diamond), 50 (dark gray triangle), or 100 (black square) % cover of oysters in mussel-beds or mud-flats. ANOVA F ratios and P values are included, mean \pm SE.

analyses were fluxes of NH_4^+ , Si(OH)_4 , CO_2 , CH_4 , and N_2O , inventories and residence times of NH_4^+ and Si(OH)_4 and content of OM, TOC, and TON. Marginal tests (that is, fitting of each variable individually, ignoring other variables) were followed by the *all specified* selection procedure with the *adjusted R^2* selection criterion. The significance of the marginal tests was determined based on 9,999 permutations of raw data (Anderson 2003). Distance-based redundancy analysis (dbrDA) was used to visualize the fitted DISTLM model (Legendre and Anderson 1999). Models were fitted using 9999 unrestricted permutations of raw data. Analyses were done on square-root transformed assemblage data to reduce the contribution of highly abundant taxa (Clarke and Warwick 2001). Functional variables were automatically normalized as part of the DISTLM procedure.

RESULTS

Organic Matter (OM) Content and C/N Ratio

OM % within mussel-beds only differed significantly at the 2–4 cm depth horizon, where plots with 50% cover had greater OM % than those with

0, 5, or 100% cover of *C. gigas* (Table S1). Within mud-flats, there were no significant differences in OM % at any depth. Although not significantly different, the OM % in the 0–2 cm depth tended to increase with increasing cover of *C. gigas* within mussel-beds (Table S1).

TOC %, TON %, and C/N ratios did not significantly differ between habitats or among different cover of *C. gigas* (Table S2). Although not significantly different, TOC and TON % in mussel-beds were greatest in plots with 50% cover of *C. gigas* and in mud-flats TOC and TON % tended to increase with increasing cover of *C. gigas* (Table S2).

Pore-water Nutrients

In mussel-beds, the mean (\pm SE) NH_4^+ concentration ($\mu\text{mol dm}^{-3}$) in plots with 50 or 100% cover of *C. gigas* ranged from 30.97 ± 15.68 to 421.79 ± 103.08 , which was significantly greater than that in plots with 0 or 5% cover (ranged from 19.20 ± 11.07 to 224.42 ± 104.77) (Figure 1). In mud-flats, the NH_4^+ concentration ranged from 2.35 ± 0.97 to 107.94 ± 19.67 but did not significantly differ among different covers of *C. gigas* (Figure 1). In the layers from the sediment–water interface to 3 cm depth within mussel-beds, NH_4^+ concentration was

Table 1. Diffusive Fluxes ($\mu\text{mol m}^{-2} \text{h}^{-1}$), Inventories ($\mu\text{mol dm}^{-3}$) and Residence Times (h) of NH_4^+ and $\text{Si}(\text{OH})_4$ in Plots with Increasing Cover of Oysters in Mussel-beds or Mud-flats and F ratios, Degrees of Freedom and P Values of Analyses of Variance (ANOVA)

	Flux		Inventory		Residence time	
	Mean	SE	Mean	SE	Mean	SE
NH_4^+						
Mussel-bed						
0%	22.82	5.20	1.42	0.11	6.97	1.66
5%	13.37	12.62	1.67	0.71	33.48	21.76
50%	35.61	6.57	2.53	0.08	7.80	1.92
100%	8.47	6.04	2.89	0.23	74.98	46.77
Mud-flat						
0%	3.09	1.27	0.49	0.01	21.68	7.66
5%	0.98	0.88	0.57	0.02	47.40	14.69
50%	14.08	3.84	0.89	0.16	4.77	1.08
100%	4.00	2.55	0.59	0.01	33.88	19.24
ANOVA						
Ha	$F_{1,16} = 11.68, P = 0.00$		$F_{1,16} = 58.78, P = 0.00$		$F_{1,16} = 0.05, P = 0.82$	
Co	$F_{3,16} = 1.53, P = 0.25$		$F_{3,16} = 4.31, P = 0.02$		$F_{3,16} = 1.25, P = 0.33$	
Ha \times Co	$F_{3,16} = 3.36, P = 0.04$		$F_{3,16} = 2.53, P = 0.09$		$F_{3,16} = 3.90, P = 0.03$	
$\text{Si}(\text{OH})_4$						
Mussel-bed						
0%	5.14	0.99	0.60	0.04	12.54	2.38
5%	3.61	1.61	0.43	0.13	19.53	5.37
50%	5.13	1.64	0.62	0.18	13.63	2.43
100%	1.92	1.38	0.60	0.07	84.20	36.87
Mud-flat						
0%	2.50	0.77	0.33	0.06	24.72	11.60
5%	1.62	0.36	0.24	0.03	15.78	1.62
50%	3.97	1.59	0.35	0.04	13.85	6.87
100%	3.72	0.46	0.38	0.04	10.37	0.68
ANOVA						
Ha	$F_{1,16} = 0.94, P = 0.35$		$F_{1,16} = 23.50, P = 0.00$		$F_{1,16} = 2.68, P = 0.12$	
Co	$F_{3,16} = 1.47, P = 0.26$		$F_{3,16} = 2.12, P = 0.14$		$F_{3,16} = 2.41, P = 0.10$	
Ha \times Co	$F_{3,16} = 1.37, P = 0.29$		$F_{3,16} = 0.19, P = 0.90$		$F_{3,16} = 3.83, P = 0.03$	

greatest in plots with 50% cover of *C. gigas*, but from the 3 to 10 cm depth NH_4^+ concentration was greatest in plots with 100% cover of *C. gigas* (Figure 1). Within mud-flats, NH_4^+ concentration was greatest within plots with 50% cover of *C. gigas* at each depth (Figure 1).

Mean (\pm SE) $\text{Si}(\text{OH})_4$ concentration ($\mu\text{mol dm}^{-3}$) was significantly greater in mussel-beds ranging from 9.12 ± 4.20 to 115.35 ± 20.41 compared to mud-flats where it ranged from 10.99 ± 2.50 to 73.92 ± 3.54 (Figure 2) and plots with 100, 50, or 0% cover had a greater mean concentration than plots with 5% cover of *C. gigas* (Figure 2). Within the layers from the sediment–water interface to the 3 cm depth of mussel-beds, plots with 50% cover of *C. gigas* had the greatest $\text{Si}(\text{OH})_4$ concentration (Figure 2). Within the shallowest layers (from 0 to 1 cm depth) of mud-flats, plots with 100% cover of *C. gigas* had the greatest $\text{Si}(\text{OH})_4$ concentration.

The average sediment–water nutrient fluxes estimated for NH_4^+ and $\text{Si}(\text{OH})_4$ were unidirectional, from the sediment pore-water into the overlying bottom water. Fluxes of NH_4^+ differed between habitats and among different covers of *C. gigas* (Table 1). In mussel-beds, fluxes of NH_4^+ were greater from plots with 50% cover than from those with 100, 5, or 0% cover of *C. gigas* (Table 1, SNK procedure). Although there were no significant differences between different covers of *C. gigas* in mud-flats, the average flux of NH_4^+ from plots with 50% cover of *C. gigas* was more than twice that estimated for plots with 100, 5, or 0% cover of *C. gigas*. The flux of $\text{Si}(\text{OH})_4$ was not significantly altered by the type of habitat or the cover of *C. gigas* (Table 1), but within mussel-beds was reduced in plots with 100% cover of *C. gigas* compared to those with 50, 5, or 0% cover of *C. gigas*. Within mud-flats, plots with 50 or 100% cover had greater

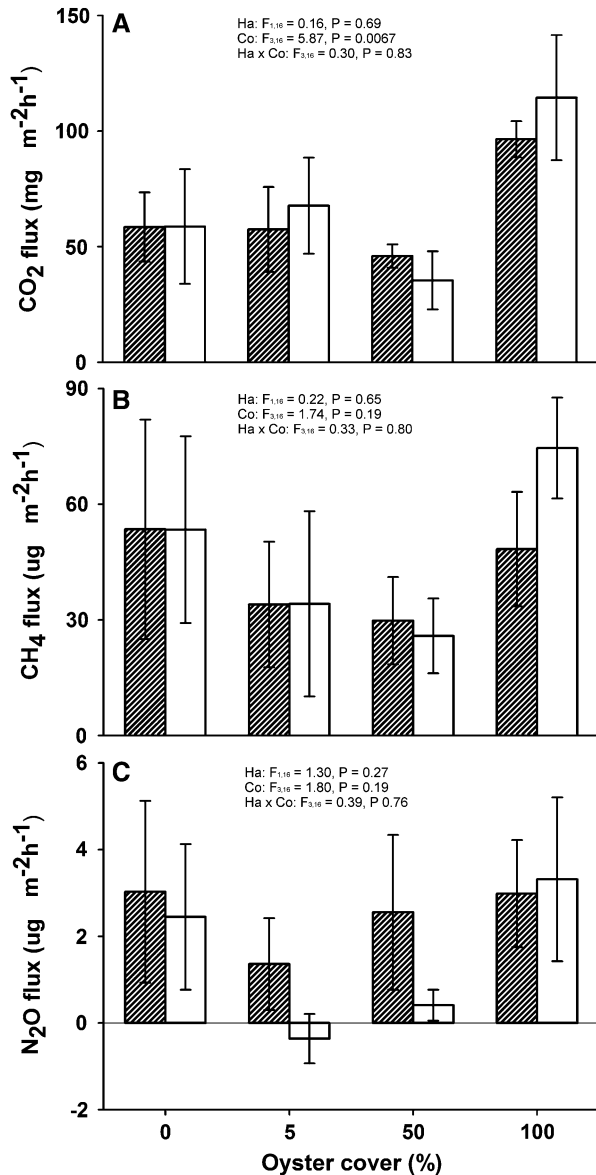


Figure 3. Sediment gas emissions. Hourly fluxes of **A** CO₂, **B** CH₄, or **C** N₂O from experimental plots with 0, 5, 50, or 100% cover of oysters in mussel-beds (shaded bars) or mud-flats (clear bars). ANOVA *F* ratios and *P* values are included, mean ± SE.

average fluxes than those with 5 or 0% cover of *C. gigas* (Table 1, SNK procedure).

The inventories of NH₄⁺ and Si(OH)₄ were greater in mussel-beds than in mud-flats (Table 1). Overall, the NH₄⁺ inventories were greater in plots with 50 or 100% cover than in plots with 5 or 0% cover of *C. gigas* (Table 1, SNK procedure). The residence time of NH₄⁺ in mussel-beds was significantly longer in plots with 100% cover than in plots with 50 or 0% cover of *C. gigas*, but in mud-flats it was significantly shorter in plots with 50%

cover than in plots with 5% cover of *C. gigas* (Table 1, SNK procedure). The residence time of Si(OH)₄ in mussel-beds was significantly longer in plots with 100% cover than in plots with 0, 5, or 50% cover of *C. gigas* (Table 1, SNK procedure) and although not significant, it was longer in plots with 0, 5, or 50% cover than in plots with 100% cover of *C. gigas* in mud-flats.

Community Respiration

CO₂ flux from the sediment was significantly affected by the cover of oysters alone, with greater fluxes in plots with 100% cover than in those with 0, 5, or 50% cover of *C. gigas* regardless of the type of habitat (Figure 3A, SNK procedure). CH₄ and N₂O fluxes did not differ significantly between habitats or among different covers of oysters (Figure 3B, C), but similar to CO₂, CH₄ fluxes tended to be greatest in plots with 100% cover of oysters.

Relationship Between Changes in Assemblage Structure and Functional Variables

Assemblage structure and diversity of macroinvertebrates and algae were altered by *C. gigas* in both habitats. In mussel-beds, plots with 100% cover differed in assemblage structure from those with 0% cover of *C. gigas* and had fewer species than plots with 0, 5, or 50% cover of *C. gigas*. Although in mud-flats, plots with 50 or 100% cover had more species and differed in assemblage structure from those with 0 or 5% cover of *C. gigas* (Green and Crowe unpublished). The functional variables together were associated with 33.1% of the total variation in assemblage structure (Figure 4). Marginal tests of variables indicated that changes in overall assemblage structure related most strongly to changes in NH₄⁺ and Si(OH)₄ inventories and NH₄⁺ flux which was associated with 18, 10, and 9% of the variation in assemblage data, respectively (Table 2).

DISCUSSION

Crassostrea gigas altered key processes involved in nutrient cycling, vital for the functioning of estuarine ecosystems, but the nature and magnitude of some of these alterations depended on the habitat and on the cover of *C. gigas*.

Oysters can increase organic matter content locally by increased deposition either directly due to their biodeposits (Newell and others 2005), or indirectly as a result of shell structures enhancing

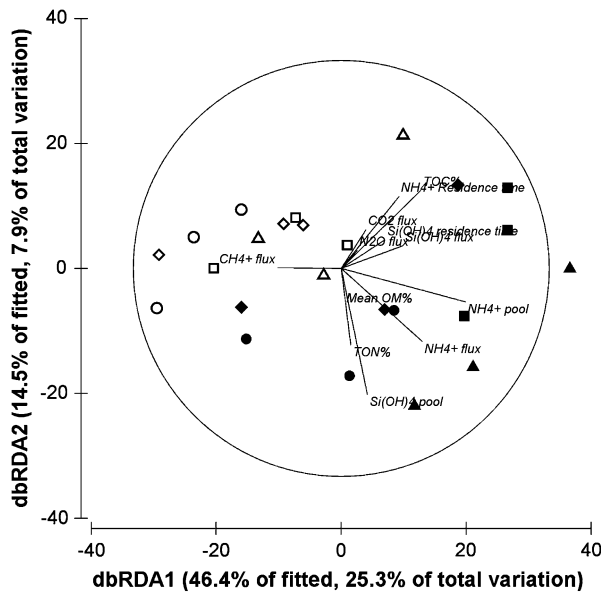


Figure 4. Relationship between functional variables and assemblage structure. Distance-based redundancy analysis (dbRDA) of square-root transformed assemblages in experimental plots with 0 (black circle), 5 (black diamond), 50 (black triangle), or 100 (black square) % cover of *C. gigas* in mussel-beds and 0 (white circle), 5 (white diamond), 50 (white triangle), or 100 (white square) % cover of *C. gigas* in mud-flats. Functional variables used to generate the dbRDA were NH_4^+ inventory, fluxes of pore-water NH_4^+ and $\text{Si}(\text{OH})_4$, gas fluxes of CO_2 , CH_4 and N_2O , average OM %, TOC and TON %.

sedimentation rates of particulate organic matter (Lenihan 1999). There was, however, only slight evidence for enrichment of total organic carbon and nitrogen in the sediment in the current study. It is possible that rather than being sequestered, the majority of additional organic matter from biodeposition was rapidly decomposed, as was evident from increased sediment carbon emissions. CO_2 fluxes increased at the highest cover of *C. gigas* similarly in each habitat, possibly due to an increase in microbial activity from the decomposition of OM supplied in biodeposits of *C. gigas* (Newell and others 2005). Although differences amongst covers were not significant, the flux of CH_4 followed a similar pattern to that of CO_2 , indicating that anoxic decomposition of OM (through methanogenesis) also increased at high cover of *C. gigas*. The combination of CO_2 and CH_4 fluxes are indicative of the total carbon catabolism in the sediment (Griffiths and others 1983), indicating that the total decomposition of organic carbon increased with high cover of *C. gigas* in both habitats. Alternatively, the increase in community respiration could have been due to respiration of

Table 2. Marginal Tests of Distance-based Linear Model (DISTLM) Analyses on Relationships between Assemblage Structure and Individual Functional Variables in Plots with Increasing Cover of Oysters in Mussel-beds and Mud-flats

Variable	Pseudo- <i>F</i>	Prop.
NH_4^+ pool	4.68	0.18***
$\text{Si}(\text{OH})_4$ pool	2.34	0.10*
NH_4^+ flux	2.06	0.09*
TOC %	2.03	0.08
TON %	1.66	0.07
$\text{Si}(\text{OH})_4$ residence time	0.91	0.04
CH_4 flux	0.91	0.04
NH_4^+ Residence time	0.75	0.03
CO_2 flux	0.72	0.03
$\text{Si}(\text{OH})_4$ flux	0.67	0.03
Mean OM %	0.66	0.03
N_2O flux	0.46	0.02

Prop. is the proportion of variance in the assemblage structure that is explained by the functional variable.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

the oysters themselves, or that of the associated macrofauna on their shells, or due to a decrease in air volume due to the oysters occupying space in the gas collection chambers. Although the current experiment cannot separate between these models, tests were made in another experiment using three procedural controls (accounting separately for oyster or macrofaunal respiration and for the differences in volume within the chambers) and differences were found to be mostly attributable to fluxes from the sediment (Green and others 2012).

The measured values of N_2O , a product of denitrification, were very low [which is not surprising because, in marine ecosystems, the flux of N_2O only represents about 5% of the total N_2 flux (Seitzinger and Nixon 1985)] and fluxes did not differ between habitats or among different covers of *C. gigas*. In contrast, NH_4^+ was affected by the cover of *C. gigas* similarly in each habitat. NH_4^+ can be directly excreted by *C. gigas* (Dame and others 1985) and associated fauna facilitated by *C. gigas*, but the main origin is likely from microbially mediated mineralization of OM which is likely to have increased in supply with increasing cover of *C. gigas* (Newell and others 2005). In each habitat, the inventories and fluxes of NH_4^+ were greatest, and the residence times were shortest, in plots with medium cover of *C. gigas*, rather than in plots with high cover. NH_4^+ may have been depleted from plots with high cover of *C. gigas*, for example, actively growing algae can intercept and assimilate NH_4^+ in the surface layers of sediment, thereby

limiting its release into the water column (Newell and others 2002). In fact, there was a significant increase in the cover of brown macroalgae, *Fucus vesiculosus*, within plots with high cover of *C. gigas* in both habitats (Green and Crowe unpublished data) and despite slow growth, *F. vesiculosus* can utilize NH_4^+ very efficiently (Pedersen and Borum 1997).

Dissolved silica [$\text{Si}(\text{OH})_4$] plays a major role in the functioning of marine ecosystems (Ragueneau and others 2002) as an essential nutrient for the growth of diatoms (a vital component of marine food webs) and so its depletion and cycling is closely tied to diatom productivity (Cohen 2003). Diatoms taken up by filter-feeders are regenerated as $\text{Si}(\text{OH})_4$ from the dissolution of biodeposits in the sediments (Ittekkot and others 2006). Filter-feeders can produce such high quantities of biodeposits that the subsequent dissolution of biogenic silica allows for high flux rates of $\text{Si}(\text{OH})_4$ and hence provide a positive feedback into primary production facilitating diatom dominance (Ragueneau and others 2002). This may be the case within mud-flats, where the residence time of $\text{Si}(\text{OH})_4$ was slightly accelerated with increasing cover of *C. gigas*, meaning that these sediments may act as an important source of $\text{Si}(\text{OH})_4$ to the water column. In mussel-beds, however, the residence time decelerated with the highest cover of *C. gigas*, possibly converting sediments into a sink for $\text{Si}(\text{OH})_4$, limiting its release into the water column. The retention of $\text{Si}(\text{OH})_4$ in sediments due to increased biodeposition has recently become a concerning consequence of invasive filter-feeding organisms (Ragueneau and others 2005). Officer and Ryther (1980) argue that phytoplankton communities can usefully be divided into those dominated by diatoms and those not. When silicate is absent, diatoms become replaced by other phytoplankton groups that do not have any requirement for $\text{Si}(\text{OH})_4$, such as dinoflagellates, which can form harmful algal blooms (Smayda 1997). These can have deleterious effects on human health (Officer and Ryther 1980) and support food webs that are not economically desirable (Chorus and Bartram 1999). Shifts of ecosystems from siliceous-based to non-siliceous-based phytoplankton communities have typically been attributed to anthropogenically induced enrichment of nitrogen and phosphorus (Rocha and others 2002). These effects, coupled with those of invasive bivalves, could lead to long-term deterioration of the water quality and economic value of this estuary.

It is important to note that diffusive fluxes calculated using Fick's law only represent diffusive mechanisms of nutrient transport and do not

include fluxes caused by advective pore-water exchange or bioturbation (Clavero and others 2000). Pore-water exchange in muddy sediments (such as in this study) are, however, more likely to be dominated by diffusion and bioturbation, rather than advection (Mermillod-Blondin 2011). Bioturbation is an important ecosystem process influencing the distribution of nutrients in sedimentary habitats (Rosenberg 2001). Bioturbating polychaetes enhance the exchange of inorganic nutrients from sediment pore-water into the overlying water (Henriksen and others 1983). There was a decrease in the abundance of infaunal polychaetes in plots with the highest cover of *C. gigas* in mussel-beds (Green and Crowe unpublished data), which may have reduced bioturbation in these plots and contributed to the reduction in $\text{Si}(\text{OH})_4$ and NH_4^+ fluxes. This same model cannot, however, explain the decrease in NH_4^+ flux in plots with high cover of oysters in mud-flats where the abundance of polychaetes was unaffected by *C. gigas* (Green and Crowe unpublished data). Community respiration can indicate microbial activity (Raina and others 2009), and was similarly affected by *C. gigas* in both habitats, suggesting that microbially mediated processes may have played the major role in determining the differences observed in benthic nitrogen cycling.

The relationship between biodiversity and ecosystem functioning has received substantial attention in recent years (Gamfeldt and Hillebrand 2008), but the effect of invasive species on this interaction has not (Sousa and others 2011), except where the effects of invader diversity have been evaluated (Byrnes and Stachowicz 2009; Isbell and Wilsey 2011). Ecosystem functioning may be affected by *C. gigas* either directly due to their biological activities, or indirectly as a result of consequent changes to biodiversity (Chapin and others 2000). Or indeed, biodiversity may be altered as a result of changes to ecosystem processes resulting directly or indirectly from *C. gigas*. The current study cannot distinguish the two as potential causal agents of the observed effects, but can highlight covariance between biodiversity and the functional properties of the ecosystems studied. Differences in inventories of NH_4^+ and $\text{Si}(\text{OH})_4$ and fluxes of NH_4^+ co-varied with the differences in assemblage structure of plots. Biodiversity (measured as species richness and Shannon-Weiner diversity) in mussel-beds followed a similar pattern to NH_4^+ and $\text{Si}(\text{OH})_4$ fluxes, increasing up until medium cover of *C. gigas* but significantly decreasing at the highest cover (Green and Crowe unpublished data). Differences in pore-water nutrient fluxes, therefore,

may have resulted from the differences in associated macrofaunal biodiversity. Alternatively, biodiversity may have responded to changes in the physical or chemical environment imposed by *C. gigas*. Even though local biodiversity is often increased by the shells of bivalves, which provide habitat and increase habitat complexity (McCoy and Bell 1991), high rates of biodeposition associated with high densities of filter-feeding bivalves might also contribute to a decrease in infaunal diversity by reducing O₂ availability at the water–sediment interface (Commito and Boncavage 1989). There was very little mortality of mussels or oysters in the experimental plots (personal observation), so there would have been a great amount of biodeposition in plots with high cover of *C. gigas*, arising from both oysters and mussels. None of these models, however, can explain the decrease in NH₄⁺ flux at the highest cover of *C. gigas* in mud-flats because here biodiversity was increased by *C. gigas*. This is not surprising because at lower densities of bivalves, such as on mud-flats, biodeposits provide an important resource for benthic species without producing unfavorable anoxic conditions and so often enhance macrofaunal diversity (Norkko and others 2001).

Few studies have explicitly examined the effects of invasive species across a gradient of invasion (Thomsen and others 2011), as most studies tend to examine “worst case scenarios”, contrasting heavily invaded with uninvaded sites. The impacts of invasive species, however, can be nonlinear with respect to invader abundance. For example, in the current study there was a marked increase in the flux of NH₄⁺ at medium cover of *C. gigas* followed by a decrease at high cover. Nonlinear dynamics can greatly complicate the extrapolation of future impacts (Parker and others 1999) and the cost of falsely assuming a linear abundance–impact relationship can be substantial (Yokomizo and others 2009).

In addition, the same densities or cover may also have different effects depending on the characteristics of the receiving environment. For example, although nitrogen cycling and community respiration were similarly affected in both habitats, effects on biodiversity and silicate cycling differed between habitats. Differences in the effects of *C. gigas* between habitats likely occurred because of pre-existing differences in the processes determining OM deposition. For example, mussel-beds are dominated by filter-feeders and so already have strong benthic–pelagic coupling and a plentiful supply of hard substrata, whereas mud-flats are dominated by passive settlement of

particulate OM and soft sediment. It is possible that different mechanisms may have operated within the different habitats, despite causing similar patterns. Our results confirm the need for more experimental studies spanning a range of habitats to assess the context-dependency of effects of invasive species (Sousa and others 2009; Thomsen and others 2011). By encompassing multiple, rather than single ecosystem processes, our findings will also contribute to emerging frameworks to map multiple ecosystem responses to multiple changes in biodiversity (for example, Gamfeldt and others 2008; Naeem and others 2009; de Bello and others 2010) to better inform management and improve conservation (Srivastava and Vellend 2005).

The importance of the effects of invasive species on ecosystem functioning has only recently been recognized (Ehrenfeld 2010), but the link between alterations to ecosystem processes and effects on ecosystem services is often overlooked (Charles and Dukes 2007; TEEB 2010). Our results show that when present at high cover, especially in mussel-beds, *C. gigas* can decrease biodiversity, reduce the efficiency of ecosystem processes such as benthic nutrient cycling and thus potentially alter regulating, supporting, and provisioning services (TEEB 2010). For example, if *C. gigas* becomes a dominant species in Lough Swilly, nutrient cycling may be altered to the extent that greater burial of OM and reduced regeneration of limiting biogenic elements reduces the carrying capacity of the estuary (Sequeira and others 2008; Cugier and others 2010), leading to a reduction of fisheries and commercial shellfish production.

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