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Impact of CO_2-induced seawater acidification on the burrowing activity of *Nereis virens* and sediment nutrient flux

S. Widdicombe*, H. R. Needham

Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth PL1 3DH, UK

ABSTRACT: A mesocosm experiment was conducted to quantify the effects of medium term (5 wk) exposure to acidified seawater on the structure of *Nereis virens* (Polychaeta) burrows and sediment nutrient fluxes. Worms were exposed to seawater acidified to a pH of 7.3, 6.5 or 5.6 using carbon dioxide (CO_2) gas. These treatments mimicked the effects of either ocean acidification (pH 7.3) or leakage from a sub-seabed CO_2 storage site (pH 6.5 and 5.6). Results from these treatments were compared to those from worms maintained in natural seawater with a pH ≈ 7.9. The experiment showed that the presence and structure of *N. virens* burrows significantly increased the sediment uptake of nitrate and the release of ammonium, nitrite and silicate. Phosphate flux was unaffected by the presence of burrows. Nutrient flux rates were also significantly affected by changes in seawater acidity. A reduction in seawater pH caused an increase in nitrate uptake and increase in ammonium release, a decrease in nitrite release and a decrease in phosphate uptake. The flux of silicate was unaffected by changes in seawater pH. As changes in acidity had no impact on the size and structure of worm burrows, it was concluded that the impact of seawater pH on nutrient flux was probably due to changes in the microbial communities responsible for nutrient transformations. Whilst this paper demonstrates that leakage from sub-seabed storage would have significant and immediate effects on nutrient cycling, impacts of ocean acidification through atmospheric absorption are less obvious. This paper concludes that ocean acidification could have a significant impact on sediment nutrient flux in coastal and shelf seas as a result of potential changes in the structure and function of bioturbating communities.

KEY WORDS: *Nereis virens* · Ocean acidification · Carbon capture and storage · Ecosystem function · Bioturbation

INTRODUCTION

Prior to the industrial revolution (ca. 1750) the atmospheric concentration of CO_2 was around 280 ppm. Since that time, a period of only 250 yr, this has increased to more than 380 ppm. This increase, caused primarily by the burning of fossil fuels and the manufacture of cement, is implicated as a primary driver of rising global temperatures and the environmental impacts associated with this 'climate change'. However, as far as Earth's oceans and the organisms therein are concerned, the biggest threat could come from another impact associated with atmospheric CO_2 increase; ocean acidification and the reduction in carbonate concentrations (Raven et al. 2005). Surface ocean pH has been maintained between 8.0 and 8.3 pH units for the last 25 million yr. However, current rates of increase in CO_2 atmospheric concentration—approximately 100 times greater than previous naturally induced increases (Blackford & Gilbert in press)—are causing seawater pH to decrease (Caldeira & Wickett 2003). These modelling results indicate that, compared with pre-industrial times, seawater pH has fallen by 0.1 pH units, indicating a 30% increase in the concentration of H^+ ions, whilst the current rate of acidification stands at 0.015 pH units per

*Email: swi@pml.ac.uk

decade (Haugan & Drange 1996). As further emissions of CO_2 are inevitable it is not unreasonable to assume that concentrations of atmospheric CO_2 will continue to rise. In the long term we face the prospect of atmospheric CO_2 levels exceeding 1500 ppm sometime between the years 2100 and 2200 (Pörtner et al. 2004), whilst the Intergovernmental Panel on Climate Change (IPCC) has predicted that levels could reach 800 ppm by 2100 (Feely et al. 2004). As a result of these increases it is possible that the pH of surface water could fall by up to 0.4 U before 2100 and a reduction of 0.7 U could occur by 2250 (Caldeira & Wickett 2003).

Political, social and environmental pressures to reduce CO_2 emissions have led governments to seek new options for CO_2 mitigation. One such option is that of geological CO_2 sequestration. This method of storage involves injecting CO_2 into underground porous reservoir rocks (Holloway 2005). The techniques required to do this are already well developed and have been in use at the Sleipner West gas field in the Norwegian sector of the North Sea since 2000 where around 1×10^6 t of CO_2 are currently being sequestered each year (Holloway 2005). Recognition of the potential of geological storage is now global, with additional storage projects being undertaken in Australia, Canada, the USA and the UK. Whilst geological storage appears to be a practical tool in reducing emissions (Gibbins et al. 2006), it is accepted that sub-seabed storage leaks are possible over time (Hawkins 2004). If leakage events from sub-seabed storage prove analogous to natural CO_2 seeps, these leaks could cause severe reductions in pH. For example, at a natural seep site located in the bay of Naples, Italy, values of seawater pH as low as 5.6 have been recorded (Hall-Spencer pers. obs.). Little is currently known about the environmental effects of low pH seawater, so data are urgently needed to quantify the potential impact of leakage on marine organisms and processes (Raven et al. 2005).

It seems inevitable that, in the near future, areas of seafloor in shallow coastal areas will be impacted by CO_2 from either atmospheric or sub seabed sources, or both. The sediments that make up the majority of seabed habitats play a crucial role in many important marine processes including the cycling of key nutrients within coastal and shelf sea ecosystems. For example, in shallow (<50 m) coastal areas up to 80% of the nitrogen required by phytoplankton may come from the bacterial regeneration of organic matter within the seabed (Dale & Prego 2002). Whilst primarily an activity untaken by microbial organisms, nutrient cycling (nutrient transformations and transport across the sediment–water interface) is significantly influenced by the activities of multicellular animals which live and feed within the sediment (e.g. Widdicombe & Austen 1998, Mortimer et al. 1999, Tuominen et al. 1999). In doing so, such species may be considered as 'ecosystem engineers' (Lawton 1994). Of particular importance are those organisms which build and irrigate permanent burrows (Fenchel 1996 and references therein). These burrows increase both the surface area of sediment across which nutrients can pass as well as the availability of sites for nutrient transformations, such as denitrification (Mayer et al. 1995). In addition, burrow irrigation/ventilation will actively transport oxygen and nutrients between the overlying water and subsurface sediments (Aller & Yingst 1978, Aller 1982). An example of such species is the polychaete worm *Nereis virens* (Sars, 1835). This species is an important burrow-building bioturbator in coastal sediments of northern temperate latitudes (Ouellette et al. 2004, and references therein) and creates a semipermanent U-shaped burrow (Bass & Brafield 1972) which it spends 20 to 30% of its time irrigating (Kristensen 1985). In doing so it has been shown to stimulate nutrient transformation processes such as denitrification (Kristensen et al. 1985) and significantly alter the flux of nutrients across the sediment–water interface (Christensen et al. 2000). *N. virens* can be locally abundant with densities as high as 2000 ind. m^{-2} (Hylleberg & Henriksen 1980) although densities between 200 and 700 ind. m^{-2} are more common (Kristensen 1984, Christensen et al. 2000). Where they occur, the burrows of *N. virens* have been shown to increase the total sediment–water interface by up to 150% (Kristensen 1984).

To date, very little is known about how sediments, the organisms they contain and the processes they support will react to changes in seawater acidity. In the light of the potential threats from both global ocean acidification and localised leakage from CO_2 storage, it is essential that the impacts of CO_2-induced acidification on sediment fauna are determined. Particular consideration should be given to ecosystem engineers that moderate key biogeochemical processes. The objectives of the current study were to use mesocosm experimentation to: (1) identify and quantify the impact of *Nereis virens* burrows on the flux of key nutrient species, and (2) determine the extent to which these impacts are affected by CO_2 induced seawater acidification.

MATERIALS AND METHODS

Sediment collection. On 20 June 2005, 80 undisturbed cores were collected from an area of moderately sorted sandy mud (median phi = 4.01) 100 m north of Plymouth Breakwater (50° 20.090' N, 4° 08.520' W); water depth was approximately 10 m. The cores were collected by sub-sampling from a 0.1 m^2 box-corer. Into each box-core sample, 9 plastic cores (10 cm dia-

meter, 20 cm long) were pushed into the sediment to a depth of 15 cm and capped. Each core was then gently removed from the box-core, sealed on the bottom with a second plastic cap and returned to the Plymouth Marine Laboratory (PML) mesocosm within a few hours of collection. Once in the mesocosm the top caps were removed and the cores were placed randomly in a system of recirculating seawater until they were transferred into the experimental set-up.

Experimental set up. On 6 July the sediment cores were placed within the experimental system and each core was individually supplied with seawater at a rate of approximately 5 ml min^{-1} using a peristaltic pump. Cores were randomly assigned to 1 of four pH treatment levels (7.9 [ambient seawater], 7.3, 6.5, 5.6). The 20 cores within each of the four pH treatments were then randomly assigned a *Nereis virens* addition treatment level: (no added worms; small worms [0.5 g]; medium worms [1.0 g]; large worms [1.5 g]; very large worms [2.0 g]) to create a 2-factor, multilevel, crossed experimental design. Each pH/*N. virens* treatment combination was replicated 4 times. Background faunal biomass at the sediment collection site was 0.22 g $core^{-1}$ wet wt (Townsend 2006). This value equated to 15% of the *N. virens* biomass in the 'small worm' treatments (1.5 g $core^{-1}$) and 4% of the *N. virens* biomass in the 'large worm' treatments (6 g $core^{-1}$).

After the cores had settled for 24 h, 3 *Nereis virens* of the appropriate size class (g wet weight) were added to each treatment core and allowed to burrow below the sediment surface. This corresponded to a density of 382 ind m^{-2}. Worms were commercially supplied ('Seabait'). To prevent the worms experiencing acidified conditions whilst out of the burrow environment, acidification of the seawater supply did not begin until 24 h after their addition. By this time all worms had burrowed beneath the sediment surface. Miron et al. (1991) found that it could take *N. virens* more than 10 d to complete a new burrow and that the burrow system may be perpetually reshaped. Consequently, any burrowing in the first 24 h would have minimal effect on the final burrow structure. Seawater pH was reduced gradually over a period of 4 d and the experiment started once the final treatment levels had been reached. The experiment ran for 5 wk during which time the supply water was monitored for pH, temperature (Table 1) and water flow. No additional food was added to the cores as this can cause excessive worm activity (Miron et al. 1991).

Seawater acidification. Carbon dioxide gas was passed through natural seawater (salinity 33.0) contained within large (450 l) reservoir tanks (Fig. 1). The CO_2 was passed through the water as very fine bubbles which enabled the CO_2 gas to pass rapidly into solution. Seawater pH in the reservoirs was monitored using flat surface, combination pH electrodes (Walchem S650CD). Once the pH had fallen to the required level, the supply of CO_2 was halted via an automated feedback relay system. As the acidified water was taken from the reservoir to supply the experimental tanks, it was replaced by natural seawater (pH ≈ 7.9) from a separate 16 m^3 seawater tank, causing the pH in the reservoir tank to increase. This increase triggered the supply of CO_2 to be restarted and CO_2 continued to bubble through the water until the pH had again been reduced to the pre-set level (Table 1). Using this method it was possible to supply large quantities of CO_2 acidified seawater of a consistent pH. The values given by the pH electrodes in the reservoir tanks were

Table 1. pH and temperature (±1 SD) of supply seawater during experimental period

pH treatment	Average pH	pH range (max.–min.)	Temperature (°C)
7.9	7.89 (± 0.02)	7.84 – 7.92	19.4 (± 0.5)
7.3	7.27 (± 0.02)	7.21 – 7.30	19.5 (± 0.5)
6.5	6.46 (± 0.07)	6.30 – 6.60	19.4 (± 0.6)
5.6	5.58 (± 0.01)	5.56 – 5.60	18.8 (± 0.4)

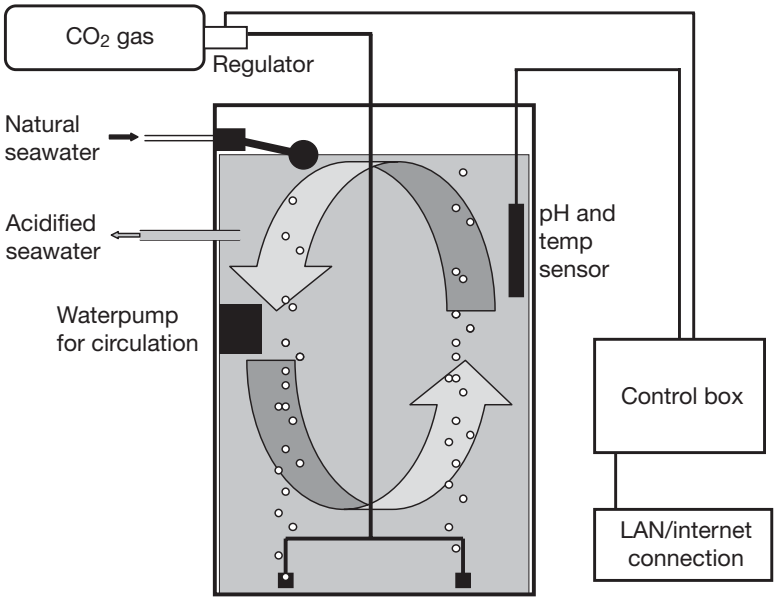

Fig. 1. Schematic diagram of seawater acidification tank

cross checked every week against values given by a regularly calibrated pH meter (InLab®413SG, Mettler-Toledo). The reservoir electrodes did not require calibrating during the course of the study.

Measurement of nutrient flux. The experiment ran for 35 d after which samples of the overlying water were taken from each core and used to determine nutrient flux. Over 3 consecutive days (15 to 17 August), three 50 ml water samples were drawn from each core, filtered through a 47 mm ∅ GF/F filter and stored in an acid washed Nalgene bottle. In addition to these 'core' samples, 15 'inflow' samples were taken from randomly selected supply tubes from each of the 4 header tanks. Each water sample was analysed by a nutrient autoanalyser (Branne & Luebbe, AAIII) for ammonium, nitrate, nitrite, silicate and phosphate concentrations using standard methods (Brewer & Riley 1965, Grasshoff 1976, Mantoura & Woodward 1983, Kirkwood 1989, Zhang & Chi 2002). Fluxes were calculated using Eq. (1) (Austen 2006).

$$F_x = \frac{(C_i - C_o) \times Q}{A} \quad (1)$$

where F_x is the flux of Nutrient x (µmol m^{-2} h^{-1}), C_i is the mean concentration of Nutrient x in the inflow water (µM), C_o is the mean concentration of Nutrient x in the core water (µM), Q is the rate of water flow through the core (l h^{-1}) and A is the area of the core (m^2). A positive flux value indicates a sediment uptake of nutrient whilst a negative value indicates a sediment release.

Measurement of burrow morphology. On 18 August 2005, 24 h after completion of the final nutrient sampling, resin casts of all burrows in each core were made by pouring approximately 500 ml of catalysed polyester resin onto the sediment surface of each core. The resin used consisted of 84% base resin, 15.5% styrene thinner and 0.5% hardener. Being denser than seawater, the resin flowed down into each of the burrows before setting. The resin at the surface of the core was hard to the touch within a matter of hours. However, the cores were then left for a further 8 d to ensure that the resin within the sediment had fully hardened. On 26 August 2005, the resin casts were removed from the sediment. This was done by removing the cap from the base of the cores and gently washing away the surrounding sediment. What remained was an intact cast of each burrow attached to a disc of the overlying resin (Fig. 2).

For each cast, measurements were taken of the maximum depth of burrow penetration and the total length of burrows. The weight of burrow casts was measured after the disc of overlying resin had been removed. From these measurements total burrow volume (Eq. 2), average burrow diameter (Eq. 3) and total burrow surface area (Eq. 4) were calculated. At the same time as the resin casts were made, random samples of the resin used were poured into 5 separate containers and allowed to set. These 5 resin samples were then cut into blocks of known volume and weighed. From this the resin density was calculated (resin density = 1.1876 g cm^{-3}).

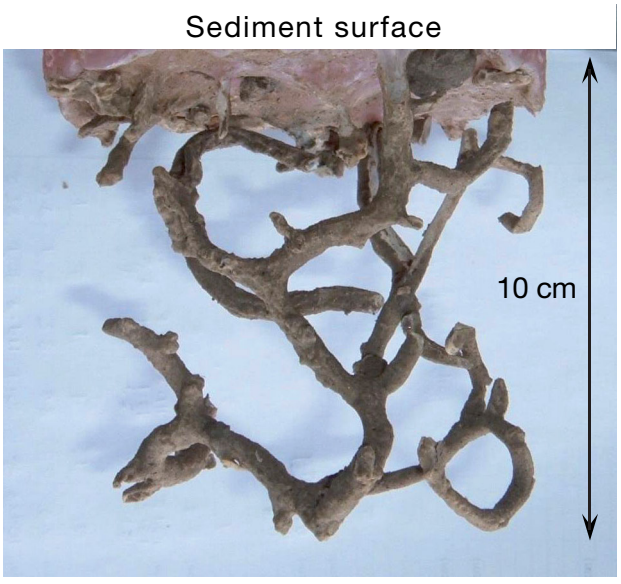

Fig. 2. *Nereis virens*. Typical resin cast of burrows

It was not always possible to differentiate between individual worm burrows within each core. Therefore, values for burrow length, volume and surface area were calculated for the total value within each core. Thus, the values presented represent the total length, volume and surface area of all burrows within the cast.

$$\text{Total burrow volume} = \frac{\text{total weight of burrow cast}}{1.1876} \quad (2)$$

$$\text{Average burrow diameter} = 2 \times \sqrt{\frac{\text{total burrow volume}}{\text{total burrow length} \times \pi}} \quad (3)$$

$$\text{Average burrow surface area} = \text{Average burrow diameter} \times \pi \text{ total burrow length} \quad (4)$$

Statistical analysis. ***Effect of pH and Nereis virens on burrow morphology:*** Microsoft Office Exel 2003 was used for data manipulations and calculations of means, CIs and SDs. Statistical analyses were conducted using Minitab 13 for Windows. A 2-way crossed analysis of variance (ANOVA) was used to test for significant effects of worm size and seawater acidity on 5 measures of burrow morphology (maximum depth, total length, average diameter, total volume and total surface area). Where significant effects were observed, mean values were obtained from the ANOVA gen-

erated table of means and 95% CIs were calculated using Eq. (5).

$$95\%\ \mathrm{CI} = t_{48}\ (5\%) \times \frac{\sigma}{4} = 2 \times \frac{\sigma}{4} \quad (5)$$

where σ is the square root of the residual mean squares from the 2-way ANOVA table.

Effect of burrows and pH on nutrient flux: As the flux of nutrients occurs across the sediment/water interface, an analysis of covariance (ANCOVA) was conducted to determine whether a significant relationship existed between burrow surface area and nutrient flux. This analysis was also used to demonstrate whether there was an overall effect of seawater pH on nutrient flux.

Prior to performing the analysis the extent of burrow surface area was calculated as a percentage of the sediment surface area (excluding burrows). This was done so as to allow direct comparisons with other studies which may have used different core sizes or worm densities.

The analyses were conducted within the general linear model function of MINITAB with the nutrient flux as the response variable, pH as the model function and burrow characteristic (e.g. burrow surface area) as the covariate. To further test whether pH had a specific effect on any relationship between nutrient flux and burrow characteristic, the ANCOVA model (H_0) was compared to the regression model (H_1):

H_0: $(y_1 = \alpha_1 + \beta x)$ $(y_2 = \alpha_2 + \beta x)$ $(y_3 = \alpha_3 + \beta x)$ $(y_4 = \alpha_4 + \beta x)$ (assumes different intercepts but a single slope for each pH treatment)

H_1: $(y_1 = \alpha_1 + \beta_1 x)$ $(y_2 = \alpha_2 + \beta_2 x)$ $(y_3 = \alpha_3 + \beta_3 x)$ $(y_4 = \alpha_4 + \beta_4 x)$ (assumes different intercepts and different slopes for each pH treatment)

The F-ratio was calculated using Eq. (6) and was referred to the distribution of F and, if $F > F$ (5%), H_0 was rejected. A rejection meant that the slopes were significantly different and therefore pH had a significant effect on the relationship between nutrient flux and burrow characteristic.

$$F = \frac{(\mathrm{RSS}_{H_0} \mathrm{RSS}_{H_1})/C}{\mathrm{RSS}_{H_1}/\mathrm{df}} \quad (6)$$

where C is the number of constraints imposed by H_0 on H_1, RSS is the residual sum of squares and df is the number of degrees of freedom of the residual under H_1.

RESULTS

Effect of seawater acidification on *Nereis virens* survival. In previous experiments using this organism (Batten & Bamber 1996, Townsend 2006) *Nereis virens* was seen to come to the sediment surface under hypoxic conditions or before death. Given that no living or dead individuals were observed at the surface during the current study it might be assumed that there was low mortality in response to changes in seawater pH. In addition, during resin casting, many worms came to the surface when resin was poured onto the sediment. On recovery of the resin casts the bodies of the worms were observed in the sediment or encapsulated in the resin. Given the relatively good condition of the bodies it would be fair to assume that most worms were alive at the time of resin casting.

Effect of seawater acidification and worm size on burrow morphology of *Nereis virens*. The size of the worms creating the burrows had a significant effect on all aspects of burrow morphology, whilst changes in pH had no significant effect on any measure of burrow morphology (Table 2). No significant interactions were observed (Table 2), which confirmed that there were no size dependent effects of pH on *Nereis virens*. As ANOVA demonstrated no significant effects of pH, values for burrow morphology can be pooled across all pH treatments (Fig. 3). Fig. 3 demonstrates that the larger the worm, the longer, wider and deeper the burrow.

Effect of burrow surface area and pH on nutrient flux. One-way ANOVA analysis confirmed that there were no significant differences between the nutrient concentrations in the inflow waters from the 4 different header tanks.

The sediment used in this experiment acted as a sink for nitrate (Fig. 4) and phosphate (Fig. 5) as well as a source of nitrite, ammonium and silicate (Fig. 4). There were significant linear relationships between burrow surface area and the flux of nitrate, nitrite, ammonium and silicate (Table 3). The numerical properties of these relationships are described in Table 4. An increase in the burrow surface area caused an increase in the sediment uptake of nitrate and a decrease in the release of nitrite, ammonium and silicate (Table 4, Fig. 4). There was no significant relationship observed between phosphate flux and burrow surface area.

Table 2. *Nereis virens*. 2-way crossed ANOVA analyses of worm size and pH effects on 5 measures of burrow morphology. (Significant values in **bold**)

Parameter	Max. depth		Length		Diameter		Volume		Surface area	
	F	p	*F*	p	*F*	p	*F*	p	*F*	p
Worm size	**9.44**	**0.000**	**4.77**	**0.005**	**44.48**	**0.000**	**33.82**	**0.000**	**22.31**	**0.000**
pH	1.84	0.153	1.70	0.180	2.41	0.078	1.72	0.176	1.62	0.198
Interaction	1.15	0.351	1.27	0.275	1.19	0.326	0.72	0.691	0.63	0.762

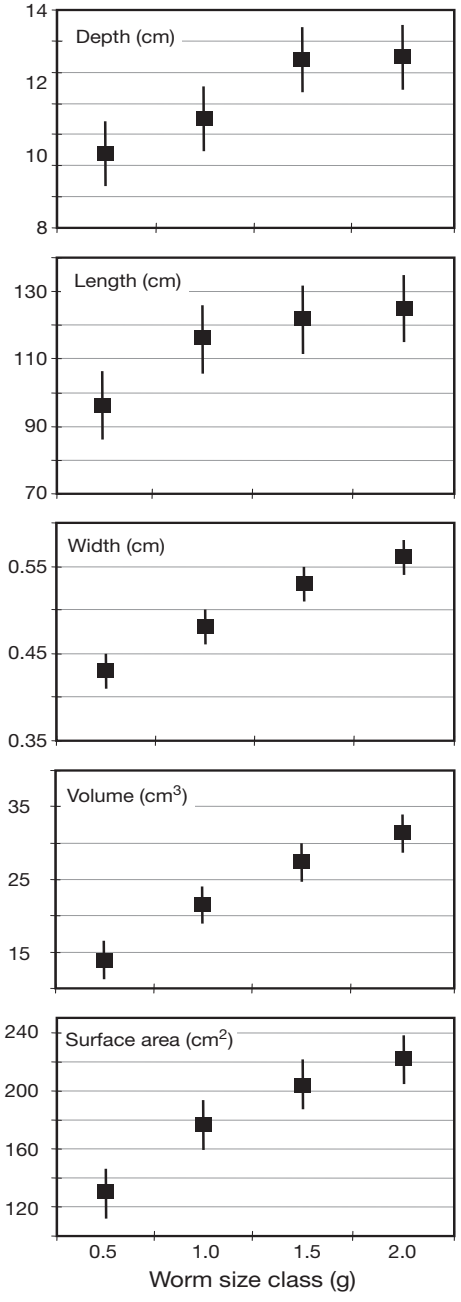

Fig. 3. *Nereis virens*. Effect of worm size on 5 measures of burrow morphology (mean ±95% CIs based on pooled SDs)

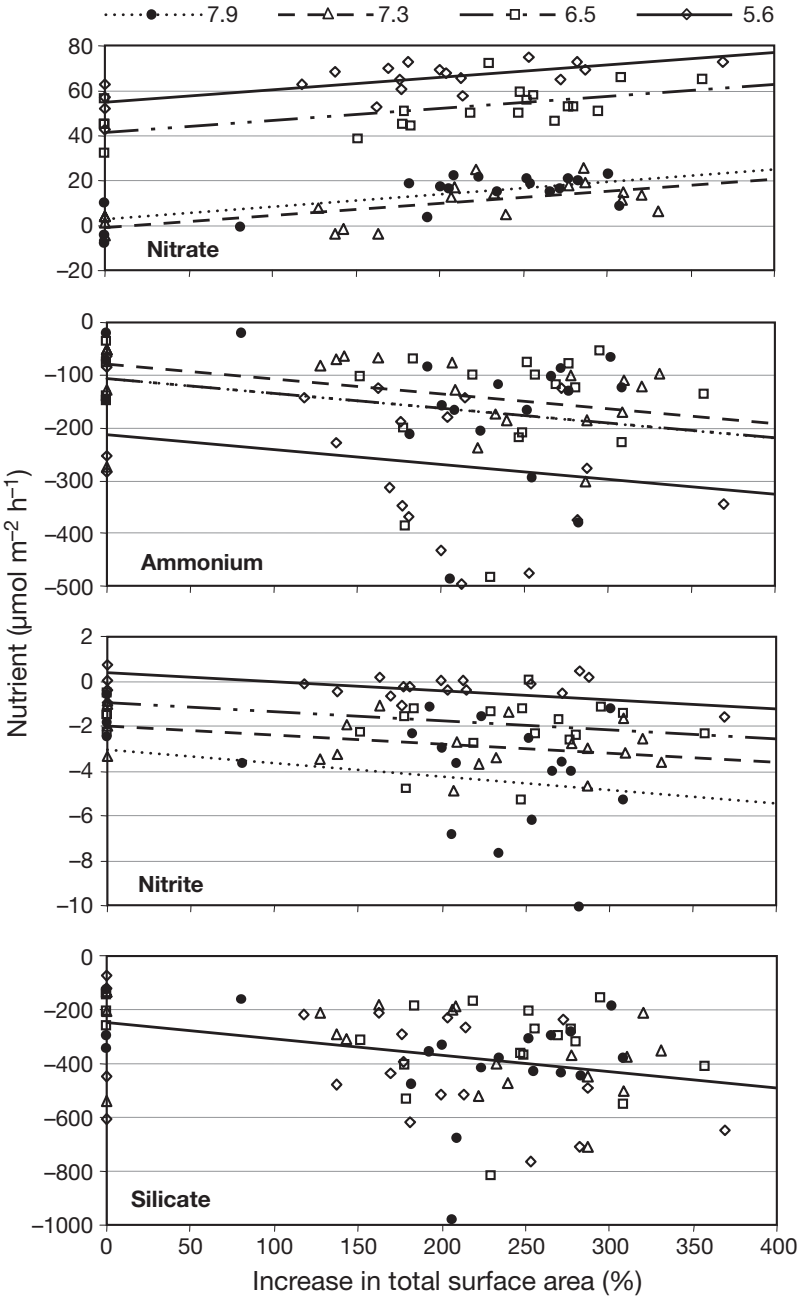

Fig. 4. Effect of seawater pH (key above panels) and *Nereis virens* burrow surface area on flux of nitrate, nitrite, ammonium and silicate

Whilst a decrease in seawater pH did affect the fluxes of nitrate (increased uptake), nitrite (decreased release) and ammonium (increased release), as demonstrated by the variations from the average intercept shown in Table 4, pH did not change the relationship (slope) between burrow surface area and the flux of these nutrients (Table 3). There was no observed effect of seawater pH on silicate flux (Table 3). Table 3 also shows a significant impact of seawater pH on the flux of phosphate.

For nitrite flux, variations from the average intercept (Table 4) indicate that changes occur when pH falls from 8.0 to 7.3. However, in the case of nitrate and ammonium much greater changes in seawater pH, between 7.3 and 6.5, were needed before a response was observed. This was also true for phosphate, for which a significant decrease in uptake by sediment was only observed when the pH was less than 7.3 (Fig. 5).

Table 3. *Nereis virens*. Impact of burrow surface area and seawater pH on nutrient flux. na: not applicable. Significant values in **bold**

Nutrient	df	Burrow surface area		pH		Slope
		F	p	*F*	p	*F*
Nitrate	3,71	**31.44**	**0.000**	**320.21**	**0.000**	0.42
Nitrite	3,69	**10.92**	**0.001**	**21.52**	**0.000**	2.118
Ammonium	3,71	**4.43**	**0.039**	**6.37**	**0.001**	0.72
Silicate	3,71	**10.42**	**0.002**	1.62	0.193	0.20
Phosphate	3,71	2.03	0.159	**14.08**	**0.000**	na

Table 4. *Nereis virens*. Numerical descriptions of burrow surface area–nutrient flux relationships for different seawater pH treatments. ns: not significant

Nutrient	Intercept	Variation from control intercept due to pH			Slope
	pH 7.9	pH 7.3	pH 6.5	pH 5.6	
Nitrate	+2.657	−3.57	+38.46	+52.15	0.055
Nitrite	−3.0092	+1.0381	+2.0821	+3.3975	−0.004
Ammonium	−105.61	+25.68	+6.41	−108.29	−0.283
Silicate	−250.12	ns	ns	ns	−0.605

DISCUSSION

Impact of *Nereis virens* burrows on nutrient flux rates

It is clear from this study that by building burrows *Nereis virens* significantly increases the area of sediment across which nutrients may pass from or into the overlying water. Our results also support the conclusions of many authors that the burrow surface area should not be considered simply as an extension of the sediment surface (Kristensen et al. 1985 and references therein). In contrast to surface sediments, the burrow environment is stable physically but not chemically (Kristensen et al. 1985 and references therein) and contains strong chemical gradients which exert significant control over biogeochemical processes. This is most readily seen in the case of nitrogen cycling, for which studies have shown that the presence of burrow builders increased the rates of both nitrification and denitrification in excess of that which would be expected purely from an increase in exchange surface area (e.g. Kristensen et al. 1985, Pelegri et al. 1994, Webb & Eyre 2004).

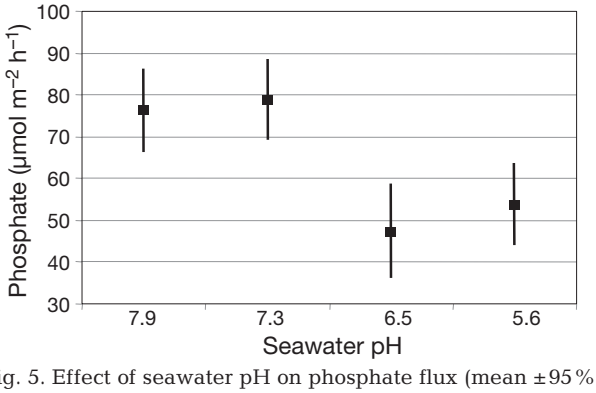

Fig. 5. Effect of seawater pH on phosphate flux (mean ±95% CIs based on pooled SD)

The nitrification potential of *Nereis virens* burrow walls is estimated to be 1.7 to 4.1 times greater than surface sediment (Kristensen et al. 1985), probably because the walls provide a more stable environment for nitrifying bacteria than surface sediment (Mayer et al. 1995). The linings of nereid burrows are made of mucus; most likely sulphated or phosphate-rich mucopolysaccharides (Kristensen et al. 1985) which attract organic particles and silt, providing an ideal substrate for microbial growth (Aller & Yingst 1978) particularly nitrifiers which, despite being chemoautotrophs, are often associated with the fine particle, high organic sediment fraction (Henriksen et al. 1983). In addition, the variable oxygen conditions within nereid burrows may induce the growth of specific strains of nitrifiers with higher activity in order to sustain sufficient growth rate (Kristensen et al. 1985). Finally, ammonium availability also plays a substantial role in the regulation of nitrification potential in burrow walls and the increase in ammonium release shown in the current study, probably as a result of excretion by *N. virens*, could have contributed significantly to the stimulation of nitrification. Given all of this it is not surprising that the sediment in the current study showed a switch from a nitrite sink in the absence of *N. virens* to a source of nitrite as the extent of *N. virens* burrows increased.

With a stimulation of nitrification it would be expected that the need for nitrate to be taken from the overlying water to fuel denitrification would be reduced. Howe et al. (2004) showed that in the case of the burrow building shrimp *Upogebia deltaura* almost 88% of the total amount of nitrate reduced via denitrification came from nitrification rather than uptake from the overlying water. This was a significantly greater percentage than for non-burrowed sediment where only 77% of the total nitrate reduced came from nitrification. However, our results demonstrate an increase in nitrate uptake as the extent of *Nereis virens* burrows increases. This could indicate that either the bacteria which convert nitrite to nitrate are in someway limited or that, more likely, the presence of *N. virens* burrows results in greater stimulation of denitrification than nitrification and a subsequent increase in the uptake of nitrate from the overlying water to fuel the increased nitrate reduction. This explanation is supported by studies which have estimated the contribution of *N. virens* burrows to bulk sediment nitrification and denitrification rates at 35% (Henriksen et al.

1980) and 50 to 79% (Kristensen et al. 1985) respectively. Further support is offered by Pelegrí et al. (1994) who showed that the burrows of the amphipod *Corophium volutator* stimulated denitrification fuelled from nitrification 3-fold, but stimulated denitrification fuelled by nitrate from the overlying water 5-fold (Pelegrí et al. 1994). In the current study, when the exchange surface area was doubled because of the presence of burrows, the increase in uptake of nitrate was twice as much as would have been expected from doubling the area of surface sediment. This would imply that any stimulation of denitrification by *N. virens* was primarily fuelled by nitrate from the overlying water. The ventilation activity of *N. virens* may also support this conclusion. Oxygen availability in burrows often limits nitrification in burrow walls and this availability is primarily controlled by the ventilation activity of the resident organism, with the duration of irrigation being more important than irrigation rate. During periods of active ventilation, oxygen levels in *N. virens* burrows are equivalent to those at the sediment surface. However, *N. virens* only spends 20 to 30% of its time ventilating its burrow and the oxygen transported into the burrow is consumed shortly (5 to 10 min) after ventilation stops (Kristensen 1985, 1989). Given this it would seem that *N. virens* burrows are primarily reducing environments, well suited to denitrification. Nitrification processes are limited to the upper few centimetres of the burrow and are likely to occur in deeper sections of the burrow only during periods of active ventilation.

The release of silicate from muddy sediments is dominated by the regeneration of biogenic silica from accumulated organic material, predominately dead and decaying diatom cells (Marinelli 1992). The current study has shown that the presence of *Nereis virens* increases the release of silicate. It is likely that, as a deposit feeder, *N. virens* consumes silica-rich sediment and therefore increases the concentration of silicate in the burrow water through the excretion of silicate-rich waste products. As the burrow is irrigated, silicate-rich water is expelled into the overlying water resulting in an increased sediment efflux. In contrast, Mortimer et al. (1999) found that the closely related species *N. diversicolor* significantly decreased the release of silicate. However, *N. diversicolor* is a suspension feeder and irrigates its burrows more actively that *N. virens*. This increased oxygenation of the burrow could result in the adsorption of silicate onto compounds such as hydrated oxides of aluminium, iron, magnesium and manganese leading to its removal from the water (Magalhães et al. 2002). This contrast between 2 closely related species highlights the importance of understanding which species build burrows but also how each species uses its burrows.

Impact of pH on *Nereis virens* activity and survival

In the current study no effects of seawater acidification were observed on *Nereis virens* mortality or on burrowing activity. Batten & Bamber (1996) however observed significantly reduced burrowing activity (number of burrows created) for *N. virens* at pH 7.5. These authors also observed mortality in worms exposed to seawater with a pH of 6.5 or less within 10 d. The results obtained by Batten & Bamber (1996) are therefore in contrast to those of the current study. The most likely explanation for this discrepancy is the medium in which the worms were retained. Batten & Bamber (1996) established *N. virens* in artificial sediment made of 1 mm glass beads whilst the current study used natural sediment. Given the known pH buffering capacity of fine sediment particles (Fenchel & Blackburn 1979) it would seem likely that the natural sediment used in the current study may have reduced the impact of seawater acidification within the burrow environment.

Batten & Bamber (1996) also reported that surviving worms displayed significant repression of growth and metabolism within 30 d at pH levels at or below 6.5. The current study did not measure growth, but it is entirely possible that whilst no impacts on mortality were observed there could have been sublethal effects. In response to hypercapnia, acid-base and ion-equilibria in many marine invertebrates will reach new steady state values. This comes at a metabolic cost and whilst not acutely life threatening is still expected to hamper growth (reduced protein biosynthesis) and reproduction during long term exposures (Langenbuch & Pörtner 2002, Pörtner et al. 2005). Some marine invertebrates are also known to produce an infusion of the neuromodulator adenosine when exposed to elevated levels of CO_2 (Reipschläger et al. 1997). This adaptive strategy, known as metabolic depression, suppresses aerobic energy turnover rate. Again, this response may be beneficial in the short term but may be detrimental to whole organism functions during long term exposure.

As no measures of growth or respiration were taken during the current study it is not possible to say categorically whether any long term metabolic depression had occurred in *Nereis virens*. However, a consequence of metabolic depression is a reduction in ammonia excretion (Pörtner et al. 2005) and it may be expected therefore that if *N. virens* had exhibited metabolic depression a reduction in the rate of ammonium release from the sediment would have been observed in response to falling seawater pH. This was not the case and, in fact, ammonium release was seen to actually increase, suggesting there was no significant suppression of metabolic processes in *N. virens*.

However, before this conclusion can be accepted it should be considered whether an increase in ventilation rate in response to hypercapnia could have caused increased burrow ventilation and therefore greater exchange between ammonium rich sediment porewaters and the overlying seawater. Such a scenario would have meant that even if metabolic depression had occurred, its impact on ammonium flux would most likely have been masked by the effects of increased burrow ventilation. Evidence from the measurements of silicate flux would seem to indicate that increased burrow irrigation had not occurred. As previously discussed, an increase in the irrigation of burrows would most likely cause a decrease in the release of silicate and, as no differences were observed for silicate flux between the different pH treatments, it may be assumed that burrow ventilation was not significantly altered by changing pH. This being the case, it is reasonable to conclude that the *N. virens*, at least within the current study, did not demonstrate large physiological responses to a medium term (5 wk) exposure to acidified seawater.

Potential experimental stress on *Nereis virens*

It should be noted that the current study is not without experimental constraints. Firstly, in the field, *Nereis virens* naturally occurs at sediment depths of between 10 and 40 cm (Andersen & Kristensen 1988), but has also been documented at depths even greater than this (Kristensen 2001). As the sediment in the current study was limited to a depth of 15 cm, a disproportionate response in the behaviour of the largest worms, which naturally burrow deeper, may have occurred. Secondly, although *N. virens* are found in very high densities naturally (Christensen et al. 2000), these values include organisms of differing life stages and therefore size classes. As each of our cores contained 3 ind. of the same weight, those cores containing the larger worms may have had a higher biomass than would naturally occur in the field even when worm density was well within natural field values. Whilst we acknowledge that these constraints could have had an influence on the results presented in this study we do not believe the influence would have been sufficient to invalidate our observations and conclusions. It should also be noted that deposit feeders such as *N. virens* are well suited to experimental systems (Berge et al. 1986) despite the obvious constraints such systems create. This is in contrast to filter feeders, such as *N. diversicolor*, for which changes in water flow and the supply of suspended material are known to affect feeding and bioturbation methods significantly (Biles et al. 2003).

Impact of seawater pH on nutrient flux

Huesemann et al. (2002) demonstrated that rates of ammonium oxidation to nitrite or nitrate (nitrification) were reduced by approximately 50% at pH 7, by more than 90% at pH 6.5, and were completely inhibited at pH 6. As the majority of nitrate used to fuel denitrification comes from nitrification rather than from the overlying water, particularly within the burrow environment, it could be predicted that a reduction in pH would cause a reduction in the supply of nitrate through nitrification and therefore a greater reliance on the uptake of nitrate from the overlying water. The results of the current study support this hypothesis in that the uptake of nitrate increases significantly as seawater pH decreases. This hypothesis assumes that the process of denitrification is less affected by pH change than nitrification and, as yet, this assumption remains to be tested.

The release of ammonium from the sediment changed very little between the controls and the treatments with seawater pH of 7.3 or 6.5. If nitrification had been reduced at a pH of ~7, it might be expected that ammonium efflux would increase. However, it is possible that instead of being released, the ammonium is oxidised anaerobically. Anaerobic ammonium oxidation (anammox) is now recognised as a significant process in the conversion of fixed nitrogen into atmospheric nitrogen (N_2) gas (den Camp et al. 2006). Recent studies have shown that this process could account for nearly 80% of the total N_2 production in some coastal sediments (Engstrom et al. 2005). The anammox process occurs when ammonia is oxidized with nitrite as the primary electron acceptor and is catalysed by a specialised group of planctomycete-like bacteria (den Camp et al. 2006). Consequently, observations of the nitrite data generated from the current study may provide indirect evidence to support the hypothesis that in acidic conditions ammonium oxidation occurs more through anammox than through nitrification. The progressive decrease in nitrite release in response to increasing pH results in nitrite uptake in non-burrowed sediment exposed to pH 5.6. This could indicate an increased demand for nitrite to fuel the anammox process. Whilst this hypothesis would seem to explain the changes observed in both ammonium and nitrite flux, the assumption that the anammox process is more tolerant to pH change than nitrification is highly speculative and remains to be tested. The dramatic increase in ammonium release at pH 5.6 was probably caused by a combination of stress-induced protein catabolism in the natural fauna within the cores together with fatalities in this natural fauna which may have stimulated bacterial production of ammonia. Cessation of the anammox process may also have contributed.

The uptake of phosphate by surface sediments occurs via assimilation by microphytobenthos (Sundbäck et al. 1991) and by adsorption of PO_4^{3-} onto hydrated metal oxides under oxic conditions (Hartikainen et al. 1996). As the anions PO_4^{3-} and SiO_4^{3-} adsorb onto the same components in the sediment and have common chemical reactions (Hartikainen et al. 1996), the lack of any pH effects on the flux of silicate would indicate that the impact of pH on phosphate flux was not due to any changes in the oxic condition of surface sediments. It is most likely therefore, that acidification of the seawater to a pH of <7.3 had a detrimental effect on surface-dwelling microphytobenthos, resulting in the observed decrease in sediment uptake of phosphate.

Implications for ocean acidification and carbon sequestration

Whilst our study has demonstrated that the effects of leakage from sub-seabed storage on nutrient cycling could be considerable, the impacts of ocean acidification through atmospheric absorption are less obvious. Predictions are that if the current rate of CO_2 release into the atmosphere continues, by the year 2100, the pH of surface oceans will be up to 0.4 U lower than at the start of the century, with a likely reduction of 0.7 U by the year 2250 (Caldeira & Wickett 2003). The current study has shown that pH changes of this magnitude are unlikely to have significant direct impacts on sediment nutrient fluxes. However, ocean acidification could have substantial indirect effects by affecting the health and activity of ecosystem engineers. Despite the high tolerance of *Nereis virens* to substantial changes in seawater pH demonstrated in the current study, this species is not the only ecosystem engineer that influences nutrient cycling. Other important bioturbating organisms could have a potentially greater vulnerability to pH change. For example, surface or shallow burrowing echinoderms are particularly vulnerable to reduced pH and hypercapnia (Spicer et al. 1988, Spicer 1995, Shirayama & Thornton 2005, Miles et al. 2007, S. Widdicombe unpubl. data), whilst being known to have significant impacts on nutrient cycling in coastal sediments (e.g. Widdicombe & Austen 1998). Differential vulnerability could therefore have a substantial effect on nutrient cycling, as the total nutrient budget represent a balance between the effects of these different types of bioturbation. Thus, in a future, more acidic ocean, an increase in the abundance of burrow building species at the expense of bulldozing bioturbators could have a significant impact on nutrient cycling in coastal and shelf seas.

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