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**The influence of
hypercapnia and
macrofauna**

H. L. Wood et al.

The influence of hypercapnia and macrofauna on sediment nutrient flux – will ocean acidification affect nutrient exchange?

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Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



Abstract

Rising levels of atmospheric carbon dioxide and the concomitant increased uptake of this by the oceans is resulting in hypercapnia-related reduction of ocean pH. Research focussed on the direct effects of these physicochemical changes on marine invertebrates has begun to improve our understanding of impacts at the level of individual physiologies. However, CO₂-related impairment of organisms' contribution to ecological or ecosystem processes has barely been addressed. The burrowing ophiuroid *Amphiura filiformis*, which has a physiology that makes it susceptible to reduced pH, plays a key role in sediment nutrient cycling by mixing and irrigating the sediment, a process known as bioturbation. Here we investigate the role of *A. filiformis* in modifying nutrient flux rates across the sediment-water boundary and the impact of CO₂-related acidification on this process. A 40 day exposure study was conducted under predicted pH scenarios from the years 2100 (pH 7.7) and 2300 (pH 7.3), plus an additional treatment of pH 6.8. This study demonstrated strong relationships between *A. filiformis* density and cycling of some nutrients; *A. filiformis* activity increases the sediment uptake of phosphate and the release of nitrite and nitrate. No relationship between *A. filiformis* density and the flux of ammonium or silicate were observed. Results also indicated that, within the timescale of this experiment, effects at the individual bioturbator level appear not to translate into reduced ecosystem influence. Rather the effect of hypercapnia and lowered pH on bacteria and microphytobenthos may have been of greater significance in understanding the changes to nutrient fluxes seen here. However, long term survival of key bioturbating species is far from assured and changes in both bioturbation and microbial processes could alter key biogeochemical processes in future, more acidic oceans.

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

1 Introduction

Increasing atmospheric levels of carbon dioxide (CO₂), resulting in ocean acidification, is recognised as a major threat to marine life (Raven et al., 2005; Widdicombe and Spicer, 2008). The consequences of increased seawater CO₂ levels are to increase both the acidity of seawater (reduce pH) and to increase its corrosiveness to calcium carbonate structures (reduced carbonate saturation level). Such changes in seawater chemistry are likely to be of particular concern in shallow coastal waters as CO₂ dissolves across the sea surface (Sabine et al., 2004). In addition, recent evidence indicates that some coastal areas are already exposed to corrosive conditions (carbonate saturated states <1) due to seasonal periods of upwelling (Feely et al., 2008). The duration and severity of these exposure periods are predicted to increase as a result of worsening ocean acidification.

Soft sediments are an important coastal benthic habitat and host many of the biogeochemical processes that underpin ecosystem function in shallow shelf seas. In particular, nutrient cycling (the “recycling” of nutrients both within and between the benthic and pelagic systems), is strongly driven by the biological and chemical processes that occur within the sediment; e.g. bacteria mineralise dissolved and particulate organic nutrients from the debris that sinks to the sea floor (Dale and Prego, 2002).

The transformation of nutrients (e.g. denitrification, nitrification and anammox) is primarily performed by bacteria and, therefore, nutrient cycling is strongly affected by the presence and activity of key microbial groups. In turn, the type and distribution of these microbes within the sediment is ultimately determined by their surrounding geochemical environment (e.g. Satoh et al., 2007). In addition to determining the microbial communities present, geochemistry can also affect nutrient flux directly. For example, the flux of silicon is dependant upon both the substrate compound availability on the sediment surface and the oxygen distribution within the sediment (Hartikainen et al., 1996). Therefore, biological processes that set or modify the geochemical nature of the sediment, such as the presence and activity of large infaunal animals, are critically

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



important for nutrient cycling.

The impacts of burrowing macrofauna on nutrient cycling are numerous (Bird et al., 1999; Christensen et al., 2000). As well as increasing the surface area of sediment available for nutrient exchange (Fenchel, 1996), it has also been suggested that the burrow itself creates a favourable environment (i.e. more than the sediment surface) for some of the bacteria involved in nutrient cycling (Henriksen et al., 1983; Kristensen et al., 1985). Burrow irrigation transfers both oxygen and nutrients from the pelagic system into the deep sediment. In addition, bioturbation, resulting from the presence and activity of macroinvertebrates, mixes the top layers of sediment and water (Eckman et al., 1981), further increasing the reaches of the sediment-water nutrient exchange, refreshing compound availability on the sediment surface and oxygenating the top layer of sediment. These cumulative effects of infaunal organisms on the sediment environment can enhance nutrient cycling directly through changing sediment geochemistry and indirectly by determining the nature and function of the resident flora and fauna (Mayer et al., 1995; Satoh et al., 2007). This habitat modification is termed ecosystem engineering (Lawton, 1994).

One important bioturbator, and ecosystem engineer, is the ophiuroid brittlestar *Amphiura filiformis*. It lives in the sediment where it suspension feeds by extending two arms into the overlying water (Loo et al., 1996). The feeding process results in the creation of a burrow environment, whereby arm undulation causes aerated water and food to move down one arm channel into a chamber where the central disk of the brittlestar is situated. Water is then forced up and out of the second arm channel. *Amphiura filiformis* occurs at high densities (often $>100 \text{ ind m}^{-2}$ but up to 3000 ind m^{-2} have been recorded, Rosenberg, 1995) and, where present, has a dominating effect on the surrounding sediment via bioturbation (O'Reilly et al., 2006). Recently, Wood et al. (2008) detected arm muscle wastage in *A. filiformis* as a result of exposure to elevated CO_2 . They speculated that arm movement and therefore bioturbation may be compromised as a result of this muscle wastage and raised the potential for key biological controls on nutrient flux, such as bioirrigation and bioturbation, to be affected by ocean acidifi-

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



cation. The current study uses a controlled laboratory experiment to expose sediment cores to CO₂ lowered pH water conditions. In addition to a set of cores run at present pH (8.0), treatments were run at pH 7.7 (expected by the end of this century), 7.3 (predicted for 2300) and a finally at pH 6.8. Several densities of *A. filiformis* were tested to determine the extent to which nutrient flux is mediated by the presence of this bioturbator and assess the degree to which this biological mediation of nutrient cycling may be altered under future scenarios of high CO₂.

2 MATERIALS and methods

2.1 Experimental set-up

Ninety undisturbed muddy-sand sediment cores (10 cm diameter, 15 cm sediment depth, 10 cm overlying water) were collected (Januar 2007) from Plymouth Sound, UK, (50°20.598 N, 4°08.155 W) using methods described in Widdicombe and Needham (2007). The cores were returned to the laboratory at Plymouth Marine Laboratory (PML) and maintained in a recirculating seawater system ($S=36$ PSU, $T=12^{\circ}\text{C}$) until required in the experiment described below. Cores that displayed evidence of burrowing activity during this time were not used. Two weeks after the collection of the sediment cores, 500 individuals of *Amphiura filiformis* (disc diameter 3–6 mm, intact and with no signs of recent regeneration), were collected from the same location. Brittlestars were taken to the laboratory at PML within 4 h of harvesting. They were kept overnight in a recirculating seawater system ($S=36$ PSU, $T=12^{\circ}\text{C}$), before visibly healthy individuals were selected for use in experiments.

Eighty sediment cores that showed no signs of animal presence (e.g. burrows) were haphazardly allocated to 1 of 4 different CO₂-acidified treatment levels (nominal pH=8.0, 7.7, 7.3, 6.8) and within each pH treatment haphazardly allocated to 1 of 5 brittlestar density levels (0, 4, 8, 12 or 16 ind core⁻¹). Each pH treatment (8.0, 7.7, 7.3 and 6.8) consisted of four replicate cores of each brittlestar density (20 cores

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



per pH treatment). Sediment cores were transferred to the PML seawater acidification facility (see Widdicombe and Needham, 2007 for details) where they were continually supplied with filtered seawater of the allocated pH at a rate 8 ml min^{-1} using a peristaltic pump. Alteration of water pH was achieved by sparging CO_2 into header tanks (vol=500 l) using the same system described by Widdicombe and Needham (2007). The exposure experiment was run for a period of 40 days.

2.2 Physico-chemical status of acidified waters

The water in each header tank, plus the water flowing out of the silicon supply tubes, was analysed three times a week for total carbon dioxide content ($t\text{CO}_2$), pH_{NIST} , salinity and temperature. $t\text{CO}_2$ was measured from $100 \mu\text{l}$ subsamples of seawater using an automated carbon dioxide analyser (CIBA Corning 965 UK). pH_{NIST} was measured using a pH electrode (Mettler Toledo LE413) calibrated with NIST standardised buffers. Salinity was measured with a conductivity salinometer (WTW LF197). Temperature was measured using a probe combined with the pH meter as detailed above (accurate to 0.1°C).

2.3 Sampling

At the end of the exposure period, a 50 ml sample of the water overlying the sediment was taken from each core. The water sample was filtered through a GFF filter and stored in an acid washed Nalgene[®] bottle. In addition a 50 ml sample of the water from each inflow tube was collected in the same way. All water samples were immediately frozen and stored frozen ($T = -20^\circ\text{C}$) to await analysis. This sampling was repeated on 3 consecutive days. Samples were analysed, after thawing, using a nutrient autoanalyser (Branne and Luebbe, AALL) for ammonium, nitrate, nitrite, silicate and phosphate concentrations using standard methods (Brewer and Riley, 1965; Grasshoff, 1976; Mantoura and Woodward, 1983; Kirkwood, 1989; Zhang and Chi, 2002). The cores were then emptied and the live *Amphiura filiformis* counted to provide survival

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



data.

2.4 Statistical analysis

Nutrient fluxes were calculated using Eq. (1) from Austen (2006), and Widdicombe and Needham (2007):

$$F_x = \frac{(C_i - C_0) \times Q}{A}, \quad (1)$$

where F_x is the flux of Nutrient x ($\mu\text{mol m}^{-2} \text{h}^{-1}$), C_i is the mean concentration of Nutrient x in the inflow water (μM), C_0 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 core area (m^2). A positive F_x value indicates the nutrient is being taken up by the sediment, and a negative value indicates nutrient being released from the sediment into the water.

Nutrient flux data were not normally distributed so were analysed using the permutational MANOVA procedures introduced by McArdle and Anderson (2001), and Anderson (2001). These procedures make more restrictive assumptions than a fully non-parametric approach, but crucially the multivariate PERMANOVA method operates on a similarity matrix and avoids unrealistic normality (or other distributional) assumptions. It does this by exploiting permutation to generate null hypothesis distributions for its pseudo-F statistics; the latter constructed by exact analogy with the standard F statistics for corresponding univariate ANOVA designs. Here, we have used the PERMANOVA+ routines (beta version, Anderson et al., 2008), which are an “add-in” to the PRIMER 6 software.

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



3 Results

3.1 Experimental conditions and *Amphiura* survival

The analysis of nutrient fluxes for cores containing no *A. filiformis* indicated that the sediment used in the current study was a sink for nitrate (104.95 ± 23.04), and a source of nitrite (-0.14 ± 0.77), ammonium (-35.01 ± 11.48), silicate (-70.58 ± 41.11) and a slight source of phosphate (-1.41 ± 0.97). Values given are means ($\mu\text{mol m}^{-2} \text{h}^{-1}$) $\pm 95\%$ confidence intervals.

The water parameters within the cores and header tanks were monitored throughout the experiment to ensure stability of the acid base-status of the water (Table 1). In all treatments pH, alkalinity, salinity and temperature remained constant throughout the experiment. Water became undersaturated with respect to calcite at pH 7.3 and with respect to aragonite at pH 7.7. The sediment in all cores appeared healthy based on the oxygenated colour of the surface and the lack of animals appearing on the surface, which is often an indication of anoxia or otherwise contaminated sediment. Three cores (one each from pH=7.7, 7.3 and 6.8 treatments) were removed from the experiment after the base caps failed during the experiment causing the water to drain out of the core resulting in air exposure and the *Amphiura filiformis* present to die. In the remaining cores *A. filiformis* survival was always 100% with feeding arms visible above the surface.

3.2 Impact of *Amphiura filiformis* on nutrient cycling

3.2.1 Nitrate, nitrite and ammonium

Amphiura filiformis presence and density did not alter ammonium flux under normo-capnic conditions (Fig. 1), while the flux of nitrite out of the sediment was significantly increased (Table 2) as *A. filiformis* density increased. The flux of nitrate from the sediment into the water column increased with density of *A. filiformis* individuals present

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



(Fig. 2).

3.2.2 Silicate and phosphate

The sediment uptake of phosphate significantly increased with increasing *Amphiura filiformis* density (Table 2), while the sediment release of silicate remained stable and unchanged by the density of *A. filiformis* (Table 2, Fig. 3).

3.3 Effect of pH on nutrient flux

A significant direct effect of pH on flux rate was only seen for nitrate (Fig. 2, Table 2) where a decreasing pH caused a reduction in the uptake of nitrate to such an extent that the sediment changed from being a sink to become a source of nitrate between pH 7.3 and pH 6.8. None of the other nutrients measured (ammonia, nitrite, silicate, and phosphate) responded directly to changes in pH (Table 2).

3.4 Impact of pH on the *Amphiura*-nutrient flux relationships

There was a significant interaction between pH and *Amphiura filiformis* density exhibited for nitrate flux (Table 2). At pH 8.0 *A. filiformis* density had no effect on the flux. However, at reduced pH where sediment uptake of nitrate was suppressed, the presence of *A. filiformis* increased the positive flux, to some extent mitigating the suppression by lowered pH. At pH=6.8 the sediment switched to become a source of nitrate to the water column; however, this nutrient loss was reduced with an increase in *A. filiformis* density (Fig. 2).

Despite no direct effect of pH or *A. filiformis* density (under normocapnic conditions) on ammonium flux rate, a significant interaction effect was identified between these two main factors (Table 2); whilst no relationship between *A. filiformis* density and ammonium flux was seen in control or pH 7.7 treatments, increasing *A. filiformis* density caused increase ammonium release from the sediment at pH 7.3 and 6.8. This indi-

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



cates an increase in the importance of *A. filiformis* bioturbation on ammonium fluxes under high CO₂ conditions.

Significant interaction effects (Table 2) indicated that increasing levels of CO₂ can intensify and/or alter the relationship between *A. filiformis* density and the sediment flux of phosphate and silicate. Phosphate flux (Fig. 3a) showed no response to *Amphiura filiformis* density at control pH, whereas at pH 7.7 the sediment uptake increased with increased *A. filiformis* density, this relationship remained but was far weaker at pH 7.3, and the trend reversed at pH 6.8 where phosphate release from the sediment was enhanced at increased *A. filiformis* density.

Silicate flux out of the sediment exhibited no discernable effect of *A. filiformis* density in the control, pH 7.7 or pH 7.3 treatments (Fig. 3b). In the pH 6.8 treatment there is a density effect, where flux of silicate out of the sediment increased with *A. filiformis* density.

4 Discussion

This study has shown that *Amphiura filiformis* can be considered as an ecosystem engineer in modifying sediment nutrient cycling. The study has also demonstrated that changing seawater pH can have a significant effect on this organism's ecosystem engineering activities by altering the relationships between *A. filiformis* density and sediment nutrient fluxes.

4.1 Impact of *Amphiura filiformis* on nutrient cycling

4.1.1 Nitrate, nitrite and ammonium

In normocapnic conditions (control pH) there was no significant effect of *Amphiura filiformis* density on nitrate flux. This could imply, therefore, that neither nitrification nor denitrification were affected, or, more likely nitrate production was enhanced through

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



burrow creation but this response was masked by a concomitant increase in nitrate sediment uptake; because the flux results obtained by the methods utilised in this study represent only net changes, an increase in both processes will therefore not be reflected in the results. The greater surface area provided by increased animal density also elucidates the significant increase in sediment nitrite release with increasing animal density; previous studies have shown burrows contain equal, and at certain depths greater, numbers of nitrite oxidising bacteria than the sediment surface (e.g. Satoh et al., 2007) thus the greater the number of *A. filiformis* and therefore burrows present in a core, the more nitrification and denitrification will occur. By the same principle ammonia-oxidising activity is likely to have increased at increased animal density (Satoh et al., 2007); while neither animal density nor pH significantly altered ammonium release from the sediment, the interaction between these factors was significant. This indicates that the biological control of ammonium flux was dependant on seawater pH.

4.1.2 Silicate and phosphate

Phosphate flux significantly changed with density of *Amphiura filiformis* switching the sediment from a source to a sink. The transport of phosphate from water to sediment can be influenced by sorption. Our results suggest that the density dependant increase in phosphate uptake results from sediment oxygenation as a result of increased bioirrigation and therefore increased oxic adsorption of phosphate ions; the oxidised burrow walls of many burrows form insoluble iron-manganese compound upon which sorption readily occurs (Aller, 1980). Indeed, for *Nereis virens*, increased worm density resulted in increased sediment P uptake (Clavero et al., 1994). It is also probable that MPB utilises some phosphate; with more surface area for the MPB and, with the presence of more burrows, this usage will also increase with animal density. Thus, sediment P uptake was influenced by burrow, and in this case *A. filiformis*, presence (but not pH), as long as these brittlestars are able to maintain their burrows. So, regardless of potential changes to irrigation rate, these phosphate data provide indirect evidence that

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



A. filiformis irrigation function is not completely halted by lowered pH, for if this were the case a pH change to phosphate sorption should have been observed.

In the current study, silicate exhibited a consistent release from the sediment; a balance between oxic precipitation and siliceous waste production by infauna. This is consistent with other publications indicating a steady release of silicate from the sediment (e.g. van der Loeff et al., 1984; Tengberg et al., 2004). Silicate flux initially appeared independent of both pH and *Amphiura filiformis* density treatments, suggesting *A. filiformis*, and its associated bioturbation and burrow creation played little role in silicate cycling. If the impact of *A. filiformis* on phosphate flux was due to oxygenation of the sediment and thus enhanced deposition then a change in silicate flux in response to changing animal density would have been expected. However, *A. filiformis* is likely to spend a proportion of its time deposit feeding on silicate rich sediment and therefore excreting silicate rich waste. This type of biological impact on silicate efflux has previously been suggested for other deposit feeders in ocean acidification studies; *Nereis virens* (Widdicombe and Needham, 2007) and *Echinocardium cordatum* (Widdicombe et al., 2009). Therefore, the net flux of silicate will be a balance between the oxic precipitation of silicate (uptake) and the excretion of silicate rich waste (release), both of which could increase with *A. filiformis* density. It is possible therefore that *A. filiformis* had a significant effect on both uptake and release processes but the overall net effect resulted in no significant change.

4.2 Effect of pH on nutrient flux

The significant effect of pH on nitrate flux was clearly visible as a reduction in sediment uptake demonstrated by the differing flux values when brittlestars were absent across pH treatments. Sediment uptake of nitrate is a process mediated by a balance between nitrifying and denitrifying bacteria. Huesmann et al. (2002) reported decreased nitrification at lowered pH with a 50% reduction at pH 7 and 90% at pH 6.5; however, while denitrification bacteria are known to be influenced by pH (e.g. Knowles, 1982; Lin and Shieh, 2006) there are few data on pH ranges of marine sediment denitrifiers-

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



most knowledge of in situ optima are derived from terrestrial soil systems (e.g. Bothe et al., 2000). Based on previous results from Widdicombe and Needham (2007), and Widdicombe et al. (2009), sediment uptake of nitrate would be expected to increase. In addition to fuelling denitrification, nitrate in sediments can also be needed to support microphytobenthos (MPB) production so the apparent decrease in nitrate uptake by the sediment in the current study could have resulted from an inhibition of MPB at low pH.

Net nitrite production in the sediment used for the current study was not affected by lowered pH over the timescale used here. In addition net ammonium flux did not change as a result of pH, indicating that either the bacterial processes that breakdown organic material and produce ammonium were not influenced by CO₂-related acidification hypercapnia, or these processes had decreased in proportion with nitrification, resulting in no net change in flux. Given the inter-relatedness of some nutrients, particularly within the nitrogen cycle, it is possible that nitrite or ammonium fluxes may have increased or decreased but if the change in one nutrient comes with a concomitant change at the next stage of the cycle, then the net change in flux will be zero.

Neither phosphate nor silicate fluxes were altered by CO₂-induced acidification alone. However it is likely that the effect of pH is hidden by the significant interaction between pH and *A. filiformis* density (Table 2) which is discussed further in Sect. 4.3. By the Redfield ratio (Redfield, 1958) principle that MPB use nitrogen and phosphate at a ratio of 17:1 (revised from the original planktonic ratio of 16:1 by Hillebrand and Sommer, 1999) then inhibition of MPB production would intuitively have a smaller impact on the flux of phosphate than on the flux of nitrate, as was the case in the current study.

4.3 Impact of pH on the *Amphiura*-nutrient flux relationships

Nitrate flux exhibited a significant interaction between pH and *Amphiura filiformis* density. In this case the presence of *A. filiformis* mitigated the suppression of nitrate uptake at lowered pH to some extent (Fig. 2); at pH 7.7, returning the sediment nitrate consumption to control levels in the highest density cores. Increased *A. filiformis* density

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



increased the positive flux of nitrate at pH 7.7 and pH 7.3. In the lowest pH treatment (pH 6.8) the flux became negative i.e. a source of nitrate to the water column. However, this release of nutrients from the sediment was reduced with increasing *A. filiformis* density. The significant positive influence on flux into the sediment (or in the case of the pH 6.8 treatment a reduction in the loss of nitrate from the sediment) could be a result of the burrow environment created by the *A. filiformis* presence; burrow irrigation and sediment bioturbation generally increases the supply of nitrate into the sediment environment where diffusive gradients cause absorption into the sediment where denitrification then occurs (Banta et al., 1999). As such, the greater the density, the more irrigation and bioturbation and therefore sediment nitrate uptake. When the slope of the density vs. flux plots are examined it is seen that the influence of the *A. filiformis* density is greater in the pH 7.7 treatment than pH 7.3 and pH 6.8; this may be due to an optimal scenario whereby increased oxygen consumption at lowered pH reduces sediment oxidisation, yet muscle wastage is minimal thus irrigation continues to supply nitrate to the sediment. Both increased oxygen consumption and arm muscle wastage are physiological responses recorded in *A. filiformis* as a result of exposure to CO₂-related acidification (Wood et al., 2008). However, the effect of muscle wastage on irrigation capability and rate requires further investigation. The significant interaction between both pH and density was attributable to the differing influence of density which had no effect on the flux at control pH, but a significant effect at lowered pH as described above. This same pattern is also seen in the significant interaction between pH and density for ammonium flux; where increasing *A. filiformis* density increases ammonium release from the sediment in the lowest two pH treatments but not at control pH or pH 7.7. While there is no significant interaction between pH and *A. filiformis* density seen in nitrite flux, it is likely, as previously mentioned, that a down regulation of both nitrite production and consumption has prevented such a change being seen when only net flux is recorded. Overall the interaction of pH and density effects on nitrate and ammonium fluxes indicate that the biological control of the nitrogen cycle nutrients across the sediment-water boundary may become even more important in

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



a high CO₂ future.

Phosphate and silicate, both of which rely on similar sediment-water exchange mechanisms, displayed a significant interaction between pH and density whereby density had no visible effect on fluxes at control pH. At pH 7.7 phosphate flux increased with increased *Amphiura filiformis* density whilst silicate flux, showed little effect of density at this level of pH. As pH decreased further no density effect is observed at pH 7.3 for either silicate or phosphate. By pH 6.8 both nutrients show a density effect, with increased *A. filiformis* density increasing the flux of both silicate and phosphate out of the sediment. The biological explanation for this response to both pH and *A. filiformis* density of phosphate flux could be the combination of changes to MPB production and *A. filiformis* function; as previously stated the increased sediment surface area in the higher density treatments, as a consequence of burrow presence, results in both a larger surface area for sorption of phosphate into the sediment and also a larger inhabitable area for MPB which means greater numbers of these algae potentially utilising phosphate. Both factors therefore explain the density dependant increase in sediment phosphate uptake seen slightly at control and more so at pH 7.7. The stronger response at pH 7.7 is probably a reflection of the previously documented (Wood et al., 2008) increased oxygen demand of *A. filiformis* at lowered pH which is expected to result in an increased rate of burrow irrigation and therefore supply of phosphate to the burrows where sorption occurs. The decreasing strength of this density response at pH 7.3 could reflect an inhibition of MPB production and therefore reduced demand for phosphate. By pH, 6.8 where sediment release of phosphate increases with *A. filiformis* density, MPB uptake of phosphate appears greatly diminished and given the documented muscle wastage within *A. filiformis* at low pH (Wood et al., 2008) it is probable that burrow irrigation, while continuing, had slowed in rate.

The flux of silicate out of the sediment showed little impact of *Amphiura* presence at control pH, 7.7 or 7.3, however at pH 6.8 the flux of this nutrient out of the sediment increased. Given that silicate release from the sediment is a balance between oxic precipitation and siliceous waste these data suggest that until pH 6.8, any increase in

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



precipitation into the sediment as a result of the burrow surface area and irrigation due to *A. filiformis* is tempered by increased siliceous waste produced by the presence of the same *A. filiformis*. This compensation in response breaks down at 6.8 whereby an increase in silicate release from the sediment is seen with increased *A. filiformis* density. As with phosphate, this suggests the rate of burrow irrigation has decreased as a result of arm muscle wastage so that while the brittlestars are still producing siliceous waste they are not facilitating oxic precipitation.

4.4 Seasonal variation and importance of bioturbating species in regulating nutrient flux

Interestingly there was no evidence of nitrification inhibition at lowered pH as observed by Huesmann et al. (2002) however, their data were derived from the response of nitrification in open water. Such work does not take into account the many contributory factors of nutrient supply and use which occur at the exchange between the sediment and overlying water. A perhaps more relevant benthic nutrient flux study into the effects of pH and macrofaunal presence by Widdicombe and Needham (2007) demonstrated a mixed response of flux to *Nereis virens* and pH, with some nutrients affected more than others. The basic sink/source properties were the same as the sediment used in this study. The impact of *N. virens* on nitrite and nitrate flux were the same as found here for *Amphiura filiformis* strengthening the concept of that ecological function i.e. burrow building was more important than the identity of the burrower. In addition, the responses of silicate and ammonium fluxes to changing pH and *A. filiformis* density were similar to the density affect of *N. virens*. But *N. virens* had no influence on phosphate uptake but both density and the interaction of pH and density affected phosphate uptake; phosphate fluxes in this earlier study were approximately 70 times greater than the current study. The most contradictory results between these similar studies however, was that of nitrate. Whereas this present study found nitrate uptake was significantly reduced by pH, Widdicombe and Needham (2007) recorded a significant increase while the burrowing animal influence alone on nitrate flux was comparable be-

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



tween the two experiments. The difference cannot be attributed to species identity as the distinctive patterns were still present with no animals present. While the sediment in the present study was from a near identical location it was collected in early January whereas Widdicombe and Needham (2007) collected their sediment in June, when temperature and nutrient supply are dramatically different. Such seasonal differences have been documented to result in a change in microphytobenthos (MPB) community structure (Hillebrand and Sommer, 1999). Consequently the different observations presented by the two studies could be due to their contrasting reliance on the MPB in driving nutrient cycling; with Widdicombe and Needham (2007) using a primarily bacterially driven system whilst the current study was more dependant on MPB activity. It is also possible that the nitrifying bacterial communities in the two studies were comprised of different species due to seasonal succession. The disparity in response, in particular with respect to nitrate, highlights the need to consider sediment nutrient flux and indeed all ecosystems in a holistic manner that incorporates, or at a minimum considers results in the context of, temporal variability and any micro-organism level changes this may incur.

5 Conclusions

The results presented here confirm that *Amphiura filiformis* are important bioturbators that affect nutrient flux. The role of *A. filiformis* in nutrient cycling is not necessarily unique; rather a function of their bioturbation and burrow irrigation activities. It is probable that high densities (often $>100 \text{ ind m}^{-2}$ but up to 3000 ind m^{-2} have been recorded, Rosenberg, 1995) and in particular constant burrow irrigation, results in their dominance as a bioturbator and ecosystem engineer in soft sediments.

The results presented here also demonstrate that CO_2 mediated changing seawater pH can have a direct effect on nutrient fluxes. Or rather that pH can influence non-macrofaunal mediated nutrient fluxes. Such results raise the importance of considering all elements of sediment-water nutrient flux facilitation; the role of microphytobenthos

BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



(MPB) was prominent in this present study, however under different conditions or in a different season, the role of bacteria facilitation may require consideration.

Physiological changes to *Amphiura filiformis* as a result of ocean acidification can impact on nutrient fluxes indirectly by changing the bioirrigatory activity of *A. filiformis* either directly through an increased demand for oxygen or food, or indirectly by muscle wastage that reduces the capacity to bioirrigate. In the case of nitrate at the intermediate altered pH of 7.7 the presence of *A. filiformis* even mitigates the effect of pH creating a situation whereby the biological control of nutrient flux is enhanced. It is still not clear whether arm movement is impaired in *A. filiformis* as a result of CO₂-related, muscle wastage, however the changes to both silicate and phosphate flux at pH 6.8, where the greatest muscle wastage is seen, support this. If muscle atrophy is ongoing, it would only be a matter of time before the brittlestar loses its feeding/irrigating ability and dies; in which case the question is can another macrofaunal burrower fill the same nutrient cycling niche? Most common bioturbators irrigate sporadically rather than continuously like *A. filiformis* and what difference this has will require further investigation.

Temporal variability is an important aspect in benthic communities and ecosystem function; the evidence presented here indicates that microphytobenthos could be of major importance in producing, and therefore understanding, some of the nutrient fluxes. This highlights the need to consider sediment nutrient flux and indeed all ecosystems in a holistic manner when investigating the impacts of ocean acidification, and in addition raises need for more experiments to inform benthic models in order to understand the long-term implications of ocean acidification at the ecosystem level.

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BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



References

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BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



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BGD

6, 2387–2413, 2009

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



The influence of hypercapnia and macrofauna

H. L. Wood et al.

Table 1. Summary of water conditions throughout experiment. Values are means \pm 95% confidence intervals.

pH treatment	measured pH	TA (mEq/L)	Sal (psu)	T ($^{\circ}\text{C}$)	Ω Calcite	Ω Aragonite
8	7.98 \pm 0.003	1726 \pm 105	36.04 \pm 0.03	11.93 \pm 0.07	1.81 \pm 0.11	1.16 \pm 0.07
7.7	7.69 \pm 0.01	2483 \pm 59	36	12.04 \pm 0.09	1.45 \pm 0.03	0.93 \pm 0.02
7.3	7.39 \pm 0.01	2214 \pm 76	36	11.97 \pm 0.07	0.53 \pm 0.02	0.34 \pm 0.01
6.8	6.76 \pm 0.04	2470 \pm 93	36.06 \pm 0.04	12.09 \pm 0.16	0.17 \pm 0.02	0.11 \pm 0.01

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)
[I◀](#)
[▶I](#)
[◀](#)
[▶](#)
[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)


Table 2. Effect of pH and *Amphiura filiformis* density on sediment nutrient flux determined using PERMANOVA analyses of two crossed, fixed factors, pH: treatment pH levels (control, 7.7, 7.3, 6.8), De: *A. filiformis* density (0, 4, 8, 12, 16 individuals per core). Type I SS given (= Type III in this case since design fully balanced). 999 permutations of residuals carried out (either under reduced or full model fitting, the outcome being the same here).

Source	df	SS	MS	Pseudo-F	P(perm)	perms
Nitrate						
pH	3	908 580	302 860	57.987	0.001	999
De	4	68 824	17 206	3.2944	0.011	998
pH×De	12	154 680	12 890	2.4681	0.008	997
Res	151	788 650	5222.9			
Total	170	1 880 800				
Nitrite						
pH	3	81.014	27.005	0.30205	0.834	998
De	4	5335.8	1333.9	14.92	0.001	995
pH×De	12	400.29	33.357	0.37311	0.966	999
Res	151	13 500	89.404			
Total	170	19 461				
Ammonium						
pH	3	25 547	8515.6	1.455	0.217	999
De	4	26 744	6685.9	1.1424	0.351	999
pH×De	12	140 870	11 740	2.0058	0.022	999
Res	151	883 750	5852.7			
Total	170	1 076 800				
Silicate						
pH	3	503 330	167 780	2.2124	0.217	999
De	4	500 620	125 160	1.6504	0.143	999
pH×De	12	1 552 900	129 410	1.7065	0.046	999
Res	151	11 451 000	75 835			
Total	170	13 785 000				
Phosphate						
pH	3	843.61	281.2	1.6292	0.182	999
De	4	5786.9	1446.7	8.3821	0.001	999
pH×De	12	5154.6	429.55	2.4887	0.009	998
Res	151	26 062	172.6			
Total	170	38 131				

The influence of hypercapnia and macrofauna

H. L. Wood et al.

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



The influence of hypercapnia and macrofauna

H. L. Wood et al.

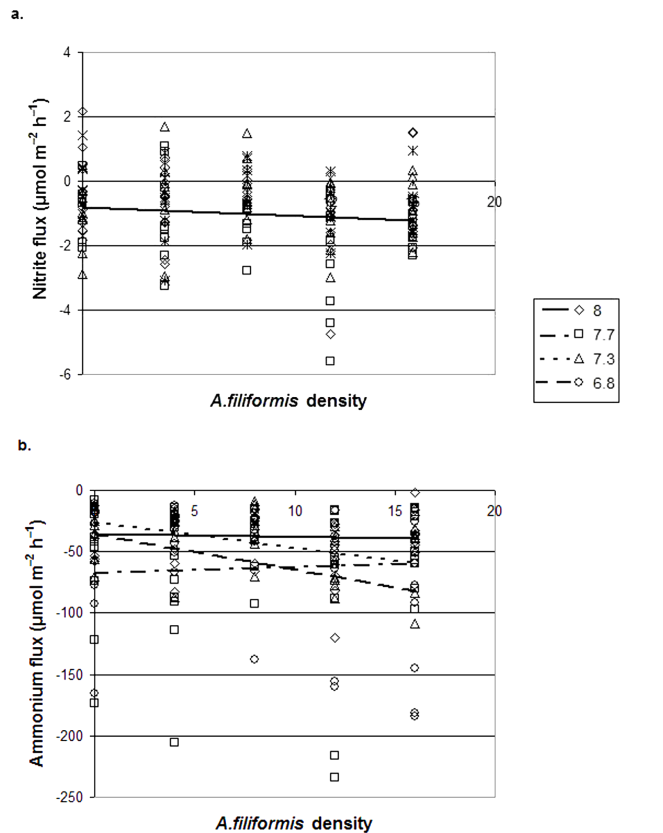


Fig. 1. Relationship between *Amphiura filiformis* density and **(a)** nitrite (line indicates best linear fit for the entire dataset) and **(b)** ammonium flux rate (best linear fit lines fitted for each pH).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion



The influence of hypercapnia and macrofauna

H. L. Wood et al.

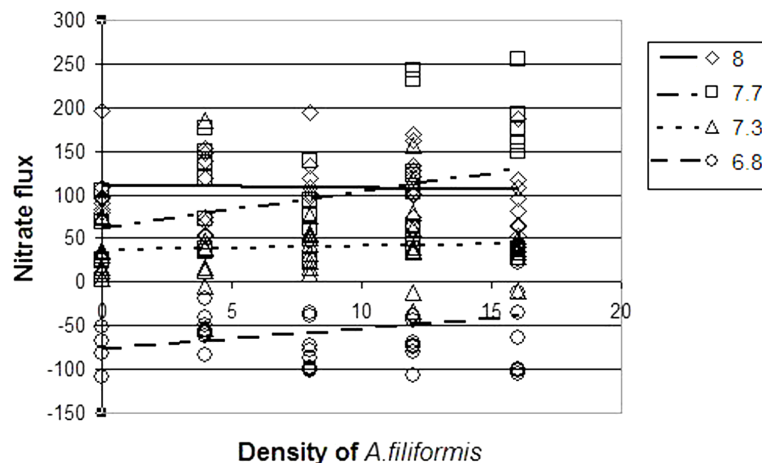


Fig. 2. Relationship between *Amphiura filiformis* density and nitrate flux (best linear fit lines fitted for each pH).

[Title Page](#)
[Abstract](#)
[Introduction](#)
[Conclusions](#)
[References](#)
[Tables](#)
[Figures](#)
[◀](#)
[▶](#)
[◀](#)
[▶](#)
[Back](#)
[Close](#)
[Full Screen / Esc](#)
[Printer-friendly Version](#)
[Interactive Discussion](#)


The influence of hypercapnia and macrofauna

H. L. Wood et al.

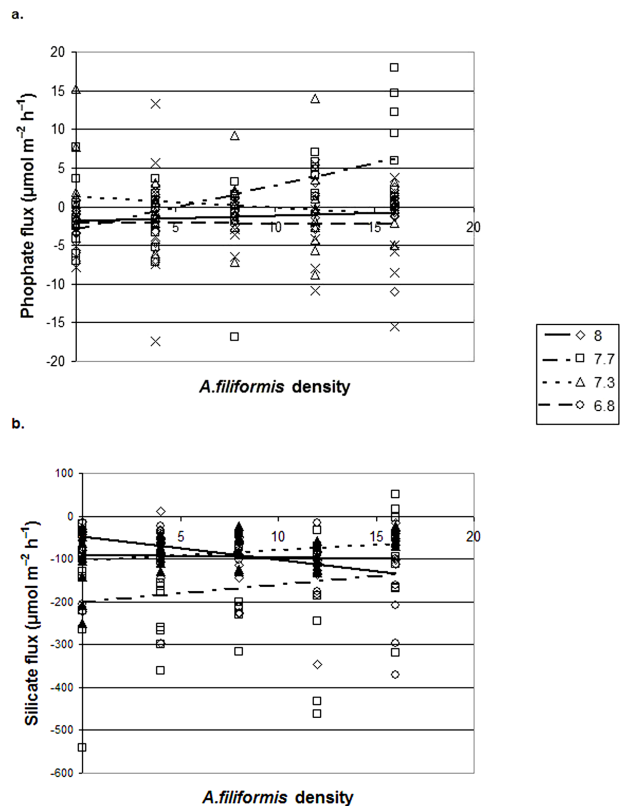


Fig. 3. Relationship between *Amphiura filiformis* density and **(a)** phosphate and **(b)** silicate flux rate (best linear fit lines fitted for each pH).

Title Page

Abstract

Introduction

Conclusions

References

Tables

Figures

◀

▶

◀

▶

Back

Close

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

