# A field test of the effects of megafaunal burrows on benthic chamber measurements of sediment-water solute fluxes

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ABSTRACT: Benthic chambers are widely used in biogeochemical studies of marine sediments, but it is not known whether sediment-water solute fluxes recorded using such apparatus will be affected by the presence of a megafaunal burrow opening in the enclosed area of sea floor. We investigated this question in a shallow sea loch using simple benthic chambers deployed by divers over 2 contrasting burrow types, those of the mud-shrimp *Callianassa subterranea* and of the echiuran worm *Maxmuelleria lankesteri*. Fluxes of dissolved oxygen, manganese (II) and nutrients were measured over an 18 to 25 h incubation period and compared with those recorded over nearby sediment lacking megafaunal burrow openings. Chambers enclosing *C. subterranea* burrows recorded a significantly higher efflux of phosphate than controls, and efflux of ammonium was also consistently (though non-significantly) greater over burrows. *C. subterranea* burrows had no detectable effect on any other measured flux. Chambers enclosing *M. lankesteri* burrow openings differed from controls only in showing a significantly higher efflux of nitrate. This nitrate effect was particularly marked over *M. lankesteri* ejecta mounds, suggesting that these features may be localized sites of enhanced nitrification. Ranges of variation were high for all measured parameters, indicating that at this site, data from a single chamber incubation would be unlikely to unambiguously signify the presence of an identifiable megafaunal burrow opening. Spatial heterogeneity in solute flux rates illustrates the need to maximize replication in field studies using benthic chambers.

KEY WORDS: Benthic chamber · Burrows · *Callianassa* · *Maxmuelleria* · Sea loch · Solute flux

## INTRODUCTION

Benthic flux chambers have become important tools for the *in situ* measurement of solute exchanges across the sediment-water interface. The technique, employing a variety of chamber designs (Malan & McLachlan 1991), has been applied both in coastal waters and in the deep ocean. Where depths are sufficiently shallow, chambers can be deployed by divers (e.g. Rutgers van der Loeff et al. 1984, Anderson et al. 1986, Barbanti et al. 1992). Chambers are also now frequently incorporated into the design of autonomous landing vehicles (Tengberg et al. 1995) and have been used in a number

of deep-sea studies (e.g. Berelson & Hammond 1986, Jahnke & Christiansen 1989, Aller et al. 1998).

Bioturbation by the benthic infauna is known to have a major influence on sediment-water fluxes of oxygen, nutrients and metals (Aller 1982, 1988), and several studies have explicitly considered the effects of faunal activity on the results obtained from benthic chamber deployments (Rutgers van der Loeff et al. 1984, Barbanti et al. 1992, Clavero et al. 1992, Forja & Gómez-Parra 1998). In these cases, attention has focused on animals in the macrofaunal size range, which are typically most abundant in the uppermost few centimetres of the sediment. However, many marine sediments also support a range of larger organisms occupying complex and long-lasting burrows which penetrate the substratum to much greater depths, extending in

extreme cases to over 3 m below the surface (Pemberton et al. 1976). The burrowing 'megafauna' includes groups such as the thalassinidean crustaceans and echiuran worms (Atkinson 1986, Nickell et al. 1995b), and it is now well-established that these large bioturbators have a powerful influence on sediment-water solute fluxes (Koike & Mukai 1983, Waslenchuk et al. 1983, Forster & Graf 1995, Ziebis et al. 1996). Megafaunal burrows are abundant in many marine sediments (e.g. Ziebis et al. 1996, Hughes & Atkinson 1997) and contribute substantially to the topographic heterogeneity of the sea floor. In environments such as these, a remotely deployed benthic chamber would have a high probability of landing over a megafaunal burrow opening, but no study has yet considered the potential of these structures to affect the solute fluxes recorded using the chamber technique.

For an accurate interpretation of spatial heterogeneity in flux rates measured using benthic chambers, it is important to determine whether fluxes obtained over megafaunal burrow openings can be distinguished from those of the 'background' sediment, and, if so, whether different burrowers have species-specific effects. We addressed this question by targeted deployments of chambers over megafaunal burrows of known identity, and by comparing the results with those obtained from control deployments over adjacent sediment lacking burrow openings.

## METHODS

**Study site and target species.** The study was carried out in Loch Sween, western Scotland, a shallow sea loch with a substratum consisting largely of organic-rich, fine muds (silt-clay content > 90 %). The study site (56° 1.4' N, 5° 36.9' W) was located approximately 150 m offshore, in a water depth of 12 to 13 m.

The muddy sediments of Loch Sween support a diverse community of burrowing megafauna (Nickell et al. 1995b). The target species, selected because of their local abundance and contrasting burrow form, were the thalassinidean mud-shrimp *Callianassa subterranea* (Montagu) and the echiuran worm *Maxmuelleria lankesteri* (Herdman). In muddy sea loch sediments *C. subterranea* constructs a complex burrow consisting of a lattice of subsurface galleries which may extend to a depth of over 80 cm, connected to the surface by 2 or more vertical shafts (Atkinson & Nash 1990, Nickell & Atkinson 1995). One surface opening is usually at the centre of a small mound of ejected sediment. In this sediment type the animal is largely a subsurface deposit-feeder (Nickell & Atkinson 1995).

The echiuran *Maxmuelleria lankesteri* inhabits a narrow, sinuous burrow in the form of an extended U

(Hughes et al. 1996). One of the 2 surface openings is at the apex of a conical or domed ejecta mound. The other opening lacks a mound and appears as a simple circular hole in the sediment surface. The burrow of this species may also reach over 80 cm depth (Nickell et al. 1995a) and typically a 60 to 80 cm horizontal distance exists between the surface openings (Hughes et al. 1999). *M. lankesteri* is a surface deposit-feeder, grazing the superficial sediment around either burrow opening with its extensible proboscis (Hughes et al. 1994).

**Benthic chamber design.** Chamber design was similar to that used on the *Elinor* benthic lander (Glud et al. 1995, 1996). The transparent polycarbonate chamber had a 30 × 30 cm square cross-section with rounded corners (Fig. 1). The basal edges were chamfered to facilitate penetration of the sediment. Total chamber height was 25 cm. A flat, removable polycarbonate lid was secured in place by 8 stainless steel clips. The edge of the lid had a rubber O-ring to ensure a good seal with the chamber. The water inside the chamber was kept circulating by a 4-bar stirrer (bar length 9 cm, diameter 1.3 cm) driven by a geared, 12 V DC motor (RS Components, Part No. 266991) held in a silicone fluid-filled housing. Power was supplied by sixteen 1.5 V industrial alkaline batteries in a sealed housing mounted in a plastic superstructure above the chamber lid (Fig. 1). The battery housing was linked to the stir-
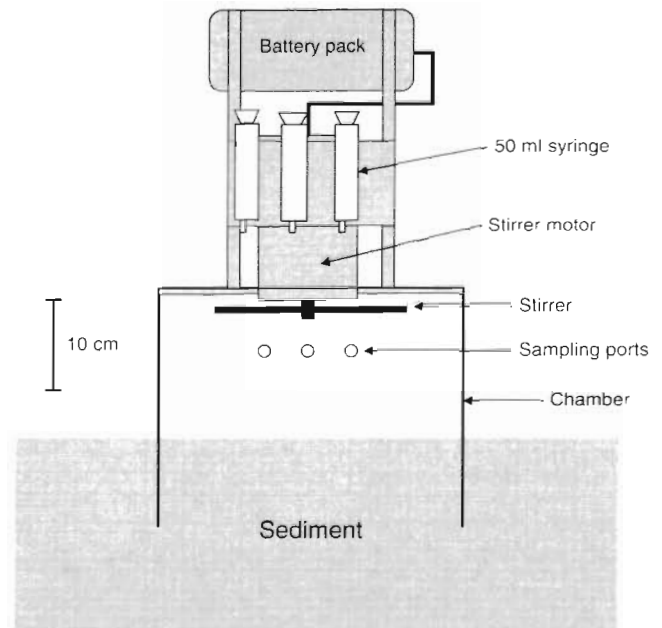


Fig. 1. Design of the diver-deployed benthic flux chambers used in this study. For clarity, only 1 set of three 50 ml syringes is shown, and the tubing connectors between the syringes and the sampling ports have been omitted

rer motor by a cable with water-resistant 3-pin connectors. The stirrer was set to rotate at 4 rpm, equivalent to approximately 5 cm s$^{-1}$. It is important to note that this figure represents the circular movement rate of the stirrer arms, not the flow velocity at the sediment-water interface, which was not measured.

The chambers were designed to be deployed by divers over selected areas of sea floor, and for the enclosed water to be sampled by manual extraction of 50 ml volumes via syringe. Three sampling ports were present in each of 3 of the chamber side walls, each port being connected to a 50 ml syringe by a short length of silicone rubber tubing (4 mm bore). The syringes were mounted in groups of 3 on panels bolted to the plastic superstructure supporting the battery housing (Fig. 1). Each group comprised 1 glass and 2 plastic syringes.

**Chamber deployment.** Five benthic chambers were available for use in this study. On-site, these were secured in lightweight metal frames and gently lowered to the sea bed from a small boat. Divers then removed the chambers from their deployment frames and detached the lid from each one. A diver then placed each chamber over a megafaunal burrow opening, or over a control area of sediment without burrow openings. Each chamber was put in place without its lid in order to minimize pressure wave-induced disturbance to the sediment surface. The 25 cm-high chambers were carefully pushed into the sediment to a depth of 10 cm, thus enclosing a water column 15 cm high. A lid was then fitted to each chamber and secured in place. The tubing connectors of the nine 50 ml syringes on each chamber were then attached to the corresponding sampling ports in the chamber sides, the motor was switched on and the stirrer set in motion.

**Water sampling.** An initial set of water samples (3 × 50 ml syringes, 1 glass, 2 plastic) was taken from within each chamber immediately after completion of the deployment procedure described above. After being filled with 50 ml of water from inside the chamber, each of the 3 syringes was disconnected from the chamber for recovery to the surface. A screw clamp on the syringe tubing was tightened to prevent any accidental loss of the sample during recovery. A port in the chamber lid, connected to 50 cm of narrow-bore tubing (to minimize diffusive exchange) allowed an inflow of external water to compensate for the volumes removed in each syringe sample. At the time of initial chamber deployment, 15 × 50 ml syringe samples (5 glass, 10 plastic) were collected external to the chambers for analysis of solute concentrations in the ambient near-bottom water. The temperature of the near-bottom water was taken from a mercury thermometer carried by a diver. Salinity was measured in the laboratory (by hydrometer) using the external water syringe samples.

The contents of each chamber were sampled again at 18 to 24 and 43 to 45 h after initial deployment. At each sampling interval 3 × 50 ml volumes of water (1 glass, 2 plastic syringes) were extracted from each chamber.

**Solute analysis.** The water samples taken from the chambers at each sampling interval were analyzed for dissolved oxygen, soluble manganese (II) (Mn$^{2+}$) and nutrients. The samples collected in glass syringes were fixed immediately on return to the shore by injection of 0.5 ml manganese (II) chloride and 0.5 ml alkaline iodide, returned to the laboratory in a sealed cool box and analyzed for dissolved oxygen by Winkler titration (Grasshoff 1983), with visual determination of the end-point. Oxygen measurements were carried out approximately 6 h after sample collection.

Water samples intended for analysis of soluble manganese (II) were passed through an in-line filter (Whatman GF/F), and a subsample was diluted 10-fold with 55 mM nitric acid. Manganese (II) concentrations in the acidified subsamples were measured by graphite furnace atomic absorption spectrophotometry (Pye Unicam Model SP9).

Samples intended for nutrient analysis were passed through an in-line filter (Whatman GF/C) and fixed by addition of 15 µl saturated mercuric chloride solution (Kirkwood 1992). Nutrient measurements were made approximately 1 wk after sample collection using a Lachat Quickchem 8000 autoanalyzer. Samples collected in 1996 were analyzed for nitrate, phosphate and silicate, those collected in 1997 were analyzed for these 3 nutrients plus ammonium.

**Solute flux calculations.** Solute flux rates were calculated using the within-chamber concentrations measured at the first 2 sampling intervals (i.e. immediately after initial deployment and after 18 to 24 h incubation). Allowance was made for the small inflow of external water that took place as each syringe sample was withdrawn from the chamber. The free water volume enclosed within a chamber was estimated using the known cross-sectional area (880 cm$^2$) and the height of the chamber above the substratum. Chamber height was nominally 15 cm, actual heights were estimated using a graduated scale (precision 0.5 cm) on the chamber side. An adjustment was made for the volume occupied by the stirrer. On each deployment, topographic irregularities (chiefly ejecta mounds) in the patch of sea floor enclosed by a chamber were noted, and their dimensions measured. Any enclosed mound was equated to the closest simple geometric solid (complete or truncated cone), its volume calculated, and the estimated water volume inside the chamber reduced accordingly.

Table 1 Temperature, salinity and dissolved oxygen concentration of near-bottom water at the Loch Sween study site during the months in which benthic chamber experiments were conducted. Oxygen values are means (±SD) of 5 replicate 50 ml water samples

| Date | Temp. (°C) | Salinity (ppt) | O$_2$ concentration (μmol l$^{-1}$) | % O$_2$ saturation |
|------|------------|----------------|-----------------------------------|-------------------|
| 14 May 96 | 9 | 35 | 205.4 ± 26.1 | 72.3 ± 8.9 |
| 11 Jun 96 | 12 | 34 | 157.5 ± 23.0 | 57.9 ± 8.5 |
| 9 Jul 96 | 13 | 34 | 179.4 ± 9.9 | 67.5 ± 3.7 |
| 6 Aug 96 | 14 | 35 | 170.4 ± 15.0 | 65.8 ± 5.8 |
| 10 Sep 96 | 14 | 34 | 97.3 ± 17.4 | 37.3 ± 6.6 |
| 11 Mar 97 | 8 | 33 | 162.1 ± 21.0 | 54.2 ± 7.0 |
| 15 Apr 97 | 9 | 35 | 165.9 ± 24.8 | 57.6 ± 8.6 |
| 10 Jun 97 | 13 | 35 | 192.5 ± 24.2 | 72.7 ± 9.1 |
| 16 Jul 97 | 14 | 35 | 177.0 ± 11.7 | 68.4 ± 4.5 |

## RESULTS

Field deployments were carried out monthly from May to September 1996, and in March, April, June and July 1997. Because of the small number of benthic chambers available, replication of results was maximized by focusing each deployment on 1 of the 2 target species. *Callianassa subterranea* burrows were studied in May to June 1996 and March to April 1997. Deployments made in July to September 1996 and June to July 1997 were devoted to *Maxmuelleria lankesteri*.

Table 1 lists the temperature, salinity and dissolved oxygen concentration of the near-bottom water at the study site during the period of each chamber experiment. Temperatures were in the range 8 to 12°C in those months in which *Callianassa subterranea* was studied, and 13 to 14°C during deployments over *Maxmuelleria lankesteri* burrows. Salinity was close to full seawater strength at all times. The near-bottom water was always hypoxic to some degree, with the lowest percentage saturation values recorded in September 1996.

### *Callianassa subterranea*

On each of the 4 deployments devoted to this species, 3 chambers were placed over *Callianassa subterranea* burrows, with 2 control chambers placed over sediment lacking surface features created by burrowing megafauna. On each occasion, the maximum distance between any of the 5 chambers was about 10 m. The criterion for selecting particular *C. subterranea* burrows for experiments was that all surface features of each target system could be enclosed within a chamber. Typically, the surface features consisted of 1 small (5 to 12 cm diameter) ejecta mound, with an adjacent pit or burrow opening present in 50% of cases. Six of the ejecta mounds had an apical burrow opening occluded by coarse, pelletized sediment. These struc-
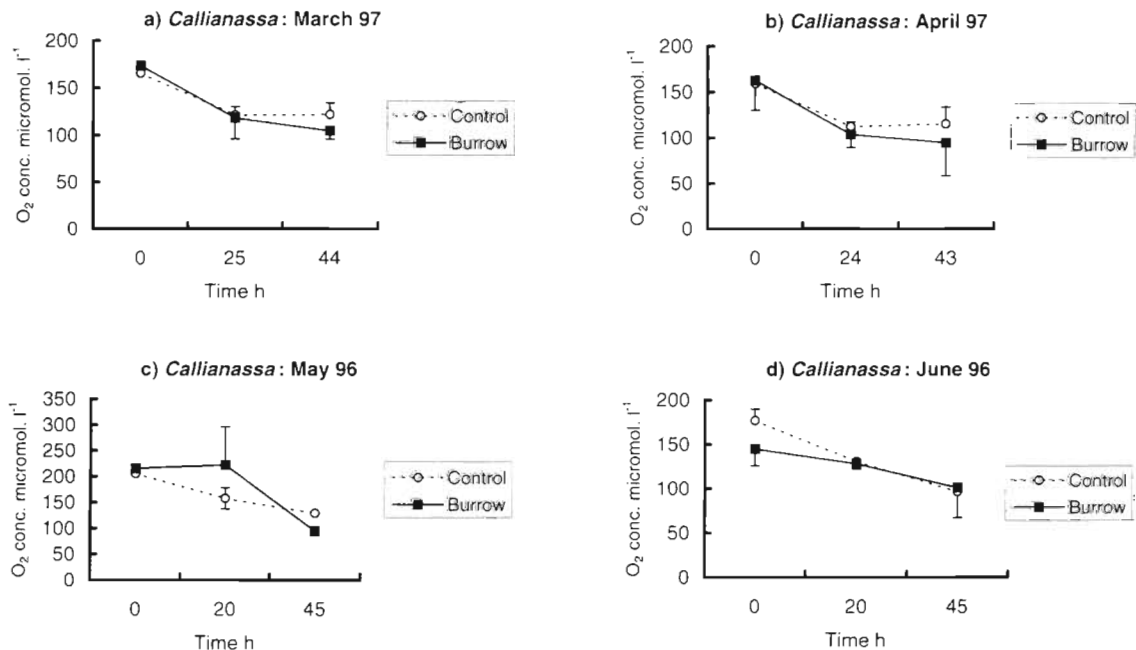


Fig. 2. Changes in dissolved oxygen concentration with time in benthic chambers placed over burrows of *Callianassa subterranea* or over nearby unburrowed sediment, in 4 separate experiments. Data points are mean values with upper or lower SD (some error bars omitted for clarity). In each experiment, n = 3 chambers over burrows, n = 2 controls
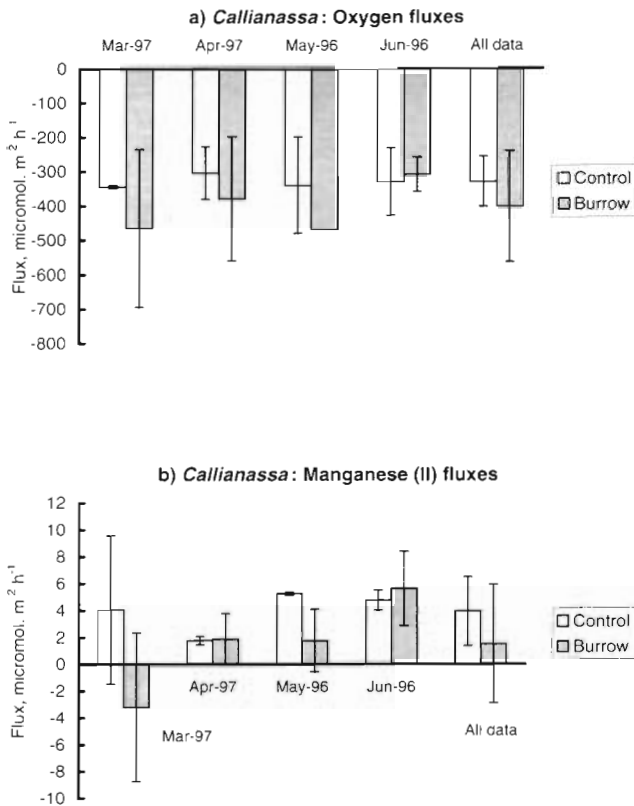
Fig. 3. Fluxes of (a) dissolved oxygen and (b) manganese (II) in benthic chambers placed over burrows of *Callianassa subterranea* or over nearby unburrowed sediment. Bars represent means ± SD Positive mean values indicate efflux from the sediment, and negative mean values uptake by the sediment. Means are presented for each individual monthly experiment, and for all data pooled. Sample sizes in each individual experiment are as outlined in Fig. 2. For pooled means, n = 8 controls, n = 12 burrows
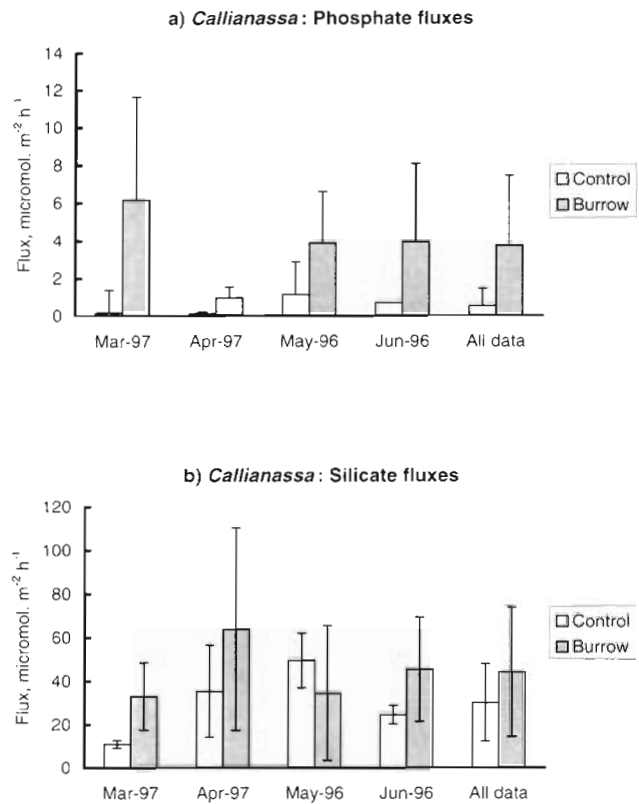
Fig. 4. Fluxes of (a) phosphate and (b) silicate in benthic chambers placed over burrows of *Callianassa subterranea* or over nearby unburrowed sediment. Bars represent means ± SD Positive mean values indicate efflux from the sediment, negative mean values uptake by the sediment. Means are presented for each individual monthly experiment, and for all data pooled. Sample sizes in each individual experiment are as outlined in Fig. 2. For pooled means, n = 8 controls, n = 12 burrows

tures were termed 'percolation mounds' by Nickell & Atkinson (1995). In no case was there any observed change in the number, size or appearance of the surface features enclosed in a chamber over the deployment period.

The enclosed water did not become anoxic in either control chambers or those deployed over *Callianassa subterranea* burrows (Fig. 2). Final dissolved oxygen concentrations (after 43 to 45 h incubation) were in the range 52.9 to 136.7 µmol l$^{-1}$, equivalent to 18.4–50.3% saturation. Over the full incubation period the change in oxygen concentration with time was strongly non-linear in at least 2 of the experiments (Fig. 2a,b), justifying the decision to use only the data from the first 20 to 25 h to calculate solute flux rates.

Mean sediment oxygen uptake rate over *Callianassa subterranea* burrows was higher than the corresponding control means in 3 of the 4 mo (Fig. 3a). The mean for burrow chambers was also higher when all data

were pooled, but the difference between this and the pooled control mean was non-significant (2-sample *t*-test, p = 0.25). Fluxes of soluble manganese (II) were highly variable, and there was no consistent trend in the differences between burrow and control means (Fig. 3b). When all data were pooled, a greater mean efflux was recorded from controls compared with burrow chambers, but the difference was non-significant (2-sample *t*-test, p = 0.14).

Mean efflux of phosphate from the sediment was always higher in chambers enclosing *Callianassa subterranea* burrows (Fig. 4a), and the difference between pooled burrow (3.74 ± 3.70 µmol m$^{-2}$ h$^{-1}$) and control (0.53 ± 0.92 µmol m$^{-2}$ h$^{-1}$) means was statistically significant (2-sample *t*-test, p < 0.05). Silicate efflux was higher over burrows in 3 of the 4 mo (Fig. 4b), but there was no significant difference between pooled means for burrow and control chambers (2-sample *t*-test, p = 0.20).

a) *Callianassa*: Nitrate fluxes

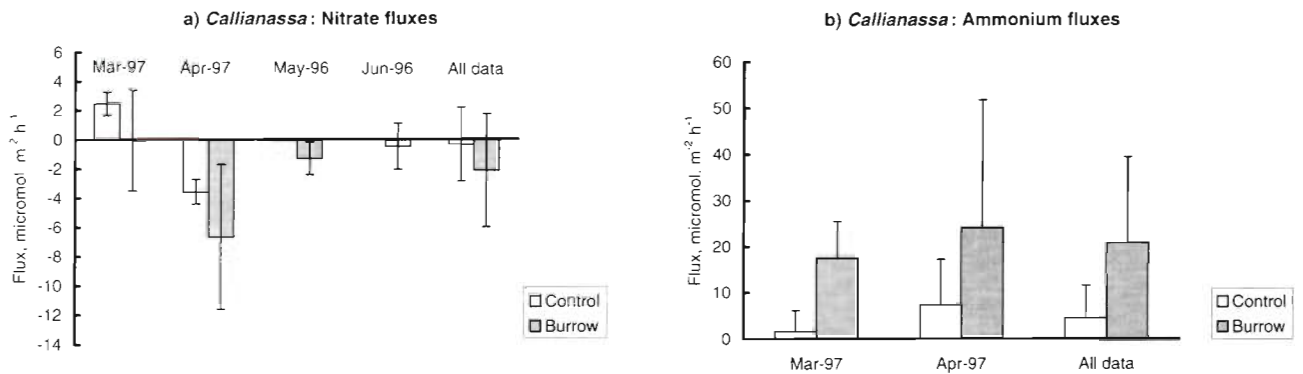b) *Callianassa*: Ammonium fluxes

Fig. 5. Fluxes of (a) nitrate and (b) ammonium in benthic chambers placed over burrows of *Callianassa subterranea* or over nearby unburrowed sediment. Bars represent means ± SD. Positive mean values indicate efflux from the sediment, negative mean values uptake by the sediment. Means are presented for each individual monthly experiment, and for all data pooled. Sample sizes in each individual experiment are as outlined in Fig. 2. For pooled nitrate means, n = 8 controls, n = 12 burrows. For pooled ammonium means, n = 4 controls, n = 6 burrows

Chambers normally recorded a strong net efflux of ammonium and a small decrease in nitrate concentration (Fig. 5). The uptake of nitrate was consistently greater in chambers enclosing burrows (Fig. 5a), but the difference from control chambers was not statistically significant when pooled means for the 2 groups were compared (2-sample *t*-test, p = 0.24). In both March and April 1997, the mean efflux of ammonium in burrow chambers was much higher than the corresponding control means (Fig. 5b). Pooling data from the 2 experiments, the burrow mean ($20.80 \pm 18.55$ µmol m$^{-2}$ h$^{-1}$) was approximately 5 times higher than the control ($4.51 \pm 7.07$ µmol m$^{-2}$ h$^{-1}$), but the variability within the burrow values (indicated by the large standard deviation of the pooled mean) meant that this difference failed to reach statistical significance (2-sample *t*-test, p = 0.10).

a) *Maxmuelleria*: June 97

b) *Maxmuelleria*: July 96 + 97

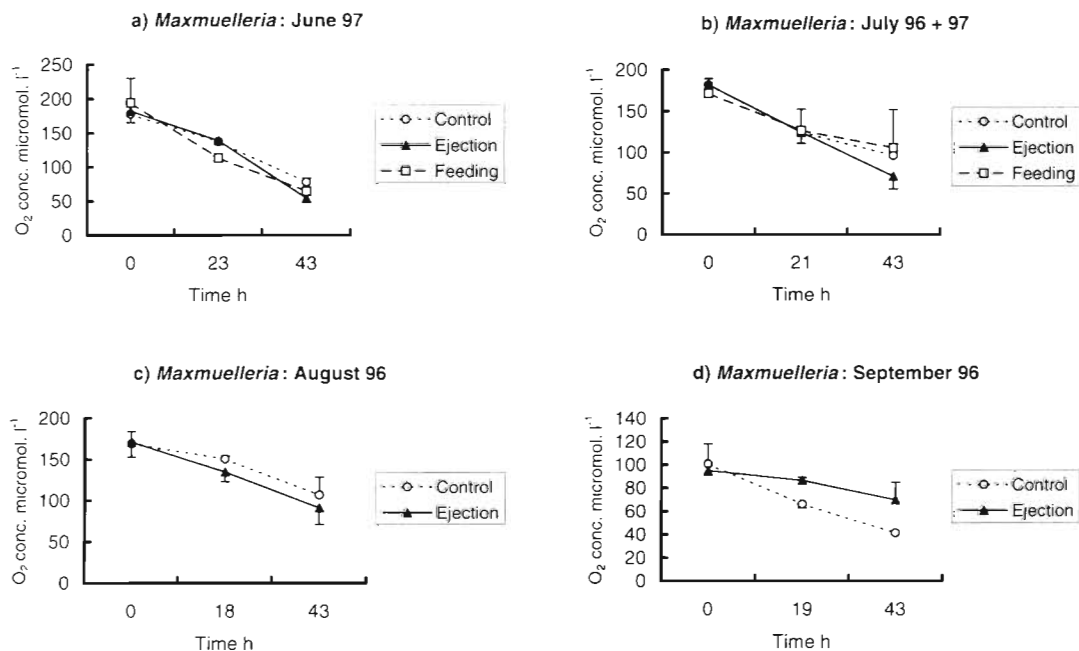c) *Maxmuelleria*: August 96

d) *Maxmuelleria*: September 96

Fig. 6. Changes in dissolved oxygen concentration with time in benthic chambers placed over burrow openings of *Maxmuelleria lankesteri* or over nearby unburrowed sediment, in 5 separate experiments, with data from July 1996 and July 1997 pooled . Data points are mean values with upper or lower SD (some error bars omitted for clarity). Sample sizes are n = 1 control, n = 2 ejection sites, n = 2 feeding sites (June 1997); n = 2 controls, n = 3 ejection sites (August 1996 and September 1996), n = 3 controls, n = 5 ejection sites, n = 2 feeding sites (July 1996 and 1997)
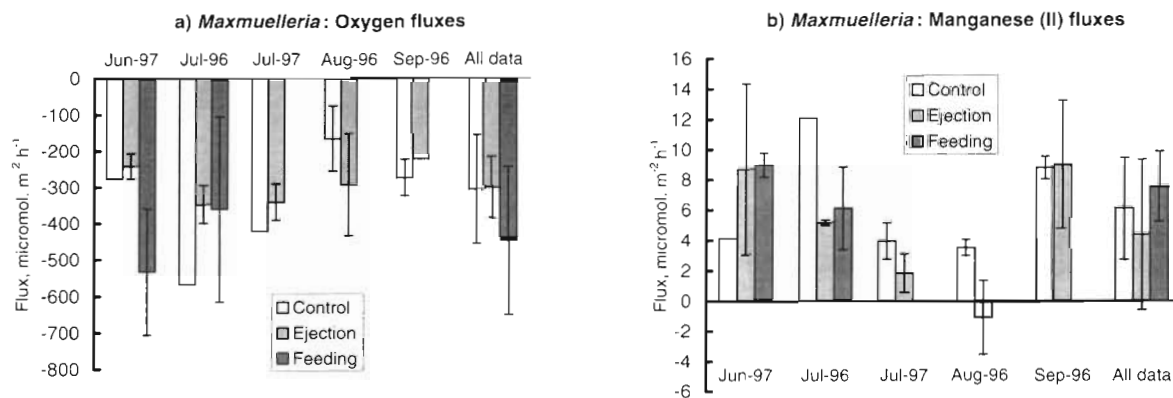
Fig. 7. Fluxes of (a) dissolved oxygen and (b) manganese (II) in benthic chambers placed over burrow openings of *Maxmuelleria lankesteri* or over nearby unburrowed sediment. Bars represent means ± SD. Positive mean values indicate efflux from the sediment, negative mean values uptake by the sediment. Means are presented for each individual monthly experiment, and for all data pooled. Sample sizes in each individual experiment are as outlined in Fig. 6. For pooled means, n = 8 controls, n = 13 ejection sites, n = 4 feeding sites

## *Maxmuelleria lankesteri*

In August 1996, September 1996 and July 1997, 3 chambers were deployed over *Maxmuelleria lankesteri* ejecta mounds, with the remaining 2 used as controls. In July 1996 and June 1997, 2 chambers were placed over ejecta mounds and 2 others over *M. lankesteri* feeding sites, with the remaining chamber in each month used as a control.

The ejecta mounds used for chamber deployments were typically 25 to 30 cm in diameter and 7 to 8 cm high in the centre, with a small (~1 cm diameter) apical burrow opening. Feeding sites lacked mounds, and with 1 exception also lacked a burrow opening at the time of the chamber deployments (the feeding openings of *Maxmuelleria lankesteri* burrows are occluded by sediment for much of the time). None of the selected mounds or feeding sites changed in appearance during the chamber incubations. In particular, no fresh material was seen to be vented from any of the ejecta mounds.

As in the *Callianassa subterranea* experiments, the chamber contents remained oxic at the end of the incubation period (Fig. 6). Final dissolved oxygen concentrations ranged from 41.0 to 140.3 µmol l$^{-1}$, equivalent to 15.7–52.7% saturation. The declines in oxygen concentration over time showed a more consistent linearity than those recorded in the *C. subterranea* experiments. However, in order to maximize comparability of results, flux rates were calculated using the data from the initial 18 to 23 h of incubation.

Comparing ejecta mounds, feeding sites and control sediment, no consistent pattern emerged in oxygen uptake rates (Fig. 7a), and the differences between pooled means were non-significant (1-way ANOVA, p = 0.17). A small efflux of soluble manganese (II) was

observed in almost all cases, but with no consistent trend in the values recorded from mounds, feeding sites and controls (Fig. 7b). Pooled means for the 3 groups were very similar and did not differ significantly (1-way ANOVA, p = 0.59). Data for phosphate (Fig. 8a) and silicate (Fig. 8b) efflux also showed no significant effects of chamber content (1-way ANOVA, p = 0.48 for phosphate, p = 0.92 for silicate).

In contrast to the *Callianassa subterranea* experiments, nitrate concentrations typically increased over the incubation period (Fig. 9a). Mean nitrate efflux rates over *Maxmuelleria lankesteri* ejecta mounds were greater than control values in all 5 experiments. Efflux rates from feeding sites in June 1997 and July 1996 fell between those for mounds and controls. When all data were considered, mean nitrate efflux rates for ejecta mounds (5.17 ± 4.20 µmol m$^{-2}$ h$^{-1}$) and feeding sites (1.87 ± 1.96 µmol m$^{-2}$ h$^{-1}$) were both higher than the control mean (0.24 ± 0.95 µmol m$^{-2}$ h$^{-1}$). The differences between the 2 burrow features and the control mean were highly significant (1-way ANOVA, p < 0.01, and Tukey's pairwise comparisons). Ammonium efflux rates were much higher in absolute terms than those for nitrate (Fig. 9b), but there were no significant differences between the 3 chamber groups (1-way ANOVA, p = 0.92).

## DISCUSSION

### Reliability of benthic chamber flux measurements

Sediment-water solute fluxes have not previously been measured in Loch Sween. Data from other Scottish sea lochs can be used to assess the reliability of the

### a) *Maxmuelleria*: Phosphate fluxes
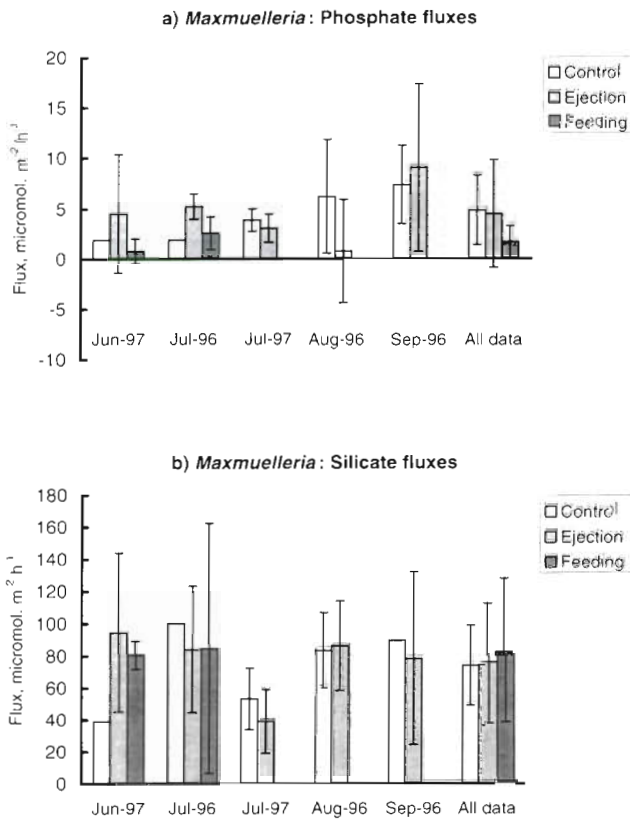


### b) *Maxmuelleria*: Silicate fluxes



Fig. 8. Fluxes of (a) phosphate and (b) silicate in benthic chambers placed over burrow openings of *Maxmuelleria lankesteri* or over nearby unburrowed sediment. Bars represent means ± SD. Positive mean values indicate efflux from the sediment, negative mean values uptake by the sediment. Means are presented for each individual monthly experiment, and for all data pooled. Sample sizes in each individual experiment are as outlined in Fig. 6. For pooled means, n = 8 controls, n = 13 ejection sites, n = 4 feeding sites

flux rates reported here, though comparisons must be made with caution given the diversity of hydrographic regimes and degrees of organic input found among these water bodies (Edwards & Sharples 1986, Howson et al. 1994). In Loch Sween, oxygen uptake rates in control chambers (i.e. avoiding any possible megafaunal burrow effects) were in the range 103.3 to 567.4 $\mu mol\ m^{-2}\ h^{-1}$, equivalent to 2.5–13.6 mmol $m^{-2}\ d^{-1}$. Overnell et al. (1995) compiled data from several sea lochs and cited uptake rates between 4.7 and 24.6 mmol $m^{-2}\ d^{-1}$, while data in the range 8 to 24 mmol $m^{-2}$ $d^{-1}$ were recorded from Lochs Goil, Fyne and Etive by Overnell et al. (1996). The Loch Sween data fall at the lower end of these published ranges but are entirely comparable in magnitude. In Loch Linnhe, nitrate showed a period of efflux during March, followed by influx in April to May and efflux again during June, while silicate efflux increased markedly after the spring

plankton bloom (Overnell et al. 1995). Both patterns were found in the Loch Sween control chamber data, and the absolute values of the fluxes were also quite comparable. Overnell et al. (1996) found that measured manganese (II) fluxes in sediment from 2 sea lochs were so variable as to raise doubts about the reliability of the data. This precludes comparison with the Loch Sween control data, which showed far less variability.

A confounding factor in the comparison of flux rates from different lochs is that most existing data are derived from laboratory core incubations rather than *in situ* benthic chamber experiments. The work of Davies (1975) in Loch Thurnaig allows a more direct comparison, although the annular chamber he used was very different to the design employed here. With an annular flow velocity of 3 cm $s^{-1}$, oxygen uptake rates in the approximate range of 130 to 780 $\mu mol\ m^{-2}\ h^{-1}$ were

### a) *Maxmuelleria*: Nitrate fluxes



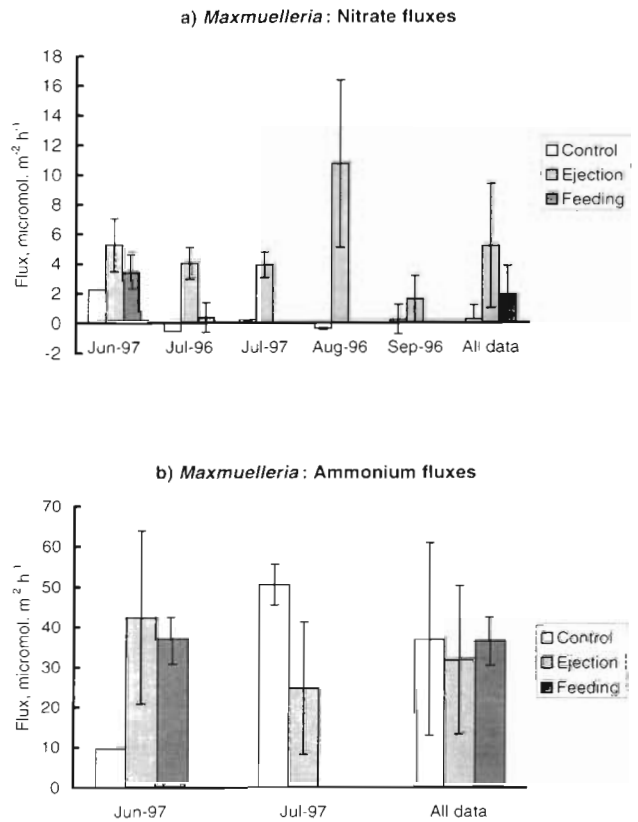### b) *Maxmuelleria*: Ammonium fluxes



Fig. 9. Fluxes of (a) nitrate and (b) ammonium in benthic chambers placed over burrow openings of *Maxmuelleria lankesteri* or over nearby unburrowed sediment. Bars represent means ± SD. Positive mean values indicate efflux from the sediment, negative mean values uptake by the sediment. Means are presented for each individual monthly experiment, and for all data pooled. Sample sizes in each individual experiment are as outlined in Fig. 6. For pooled nitrate means, n = 8 controls, n = 13 ejection sites, n = 4 feeding sites. For pooled ammonium means, n = 3 controls, n = 5 ejection sites, n = 2 feeding sites

recorded, figures very close to those obtained in Loch Sween. Ammonium efflux in Loch Thurnaig rose slightly from March to June, then showed a very steep increase in July. The same pattern was seen in the control data from Loch Sween, although the absolute values for ammonium flux were much higher (e.g. in July, approximately 5.60 $\mu$mol m$^{-2}$ h$^{-1}$ in Loch Thurnaig, compared with 50.58 $\mu$mol m$^{-2}$ h$^{-1}$ in Loch Sween).

Although caution is required in comparing data from different sea lochs, obtained using different experimental techniques, the figures presented here for Loch Sween 'background' sediment (i.e. lacking megafaunal burrow openings) are sufficiently similar to those reported from other sea lochs as to provide no grounds for doubting their reliability.

## Heterogeneity of within-treatment means

Disregarding for the moment any potential additive effects of megafaunal burrow openings, a number of factors will contribute to the wide ranges of variation associated with the mean values for each treatment group. Small sample sizes (maximum 3 chambers per treatment in each deployment) were imposed by the number of chambers available. Error bars for mean fluxes pooled across deployments are wide because the values are averaged across monthly changes in temperature and other environmental factors. Field observation of the depth of chamber penetration allowed estimation of free water volume with a relatively coarse precision of 1.0 cm$^3$, so that some measurement error was involved in the calculation of solute concentrations. Finally, all chamber flux measurements, whether over megafaunal burrow openings or 'background' sediment, include the contribution of the sediment macrofauna, which undoubtedly has a major influence on sediment-water exchanges (Aller 1982). A detailed survey of spatial patchiness in the macrofauna of the study site was beyond the scope of this work, but this factor may also explain some of the variability within treatment groups in each deployment.

## Effects of megafaunal burrow openings on measured solute fluxes

### Callianassa subterranea

Enclosed *Callianassa subterranea* burrows had no statistically significant effect on measured fluxes of oxygen, manganese (II), silicate or nitrate. *C. subterranea* burrows were associated with a significantly increased efflux of phosphate and also with a consistently increased efflux of ammonium, although the

effects on this last parameter did not reach statistical significance.

Nickell (1992) recorded enhanced concentrations of ammonium and phosphate in the water filling burrows of *Callianassa subterranea* in sea loch sediments. Ammonium and phosphate enrichment also occurs in the burrows of some other thalassinidean species (Koike & Mukai 1983, Waslenchuk et al. 1983), although Ziebis et al. (1996) found that passive advective ventilation of *C. truncata* burrows in a sandy sediment was sufficiently intense to prevent build-up of ammonium. Enclosure of all surface openings within a benthic chamber would not prevent active ventilation of the burrow by its occupant, so that Nickell's (1992) findings are in accord with the results presented here. The mean burrow irrigation rate of *C. subterranea* in aquaria was estimated as 36.3 cm$^3$ h$^{-1}$ (Nickell 1992). It is uncertain to what extent aquarium measurements are representative of activity in nature, but at this rate an animal would circulate only about 6.6% of the water enclosed in the chamber (approx. 13 200 cm$^3$) through its burrow during a 24 h experimental period. Active ventilation by the occupant may have been supplemented (or exceeded in importance) by passive flushing of the burrow induced by the chamber stirring process. The internal hydrodynamics of the chamber design used in this study (30 × 30 cm square cross-section with rounded corners, stirred by 4 cylindrical rods) have been intensively studied, with much effort devoted to measuring the effects of stirring rate on the distribution of radial pressure gradients and the thickness of the diffusive boundary layer at the sediment-water interface (Buchholtz-ten Brink et al. 1989, Huettel & Gust 1992, Glud et al. 1995, 1996). Stirring-induced advective efflux of solutes from the centre of the enclosed area is likely to be negligible in clay-rich, impermeable muds such as those of Loch Sween (Huettel & Gust 1992, Glud et al. 1995). However, the radial pressure gradient is likely to cause increased flushing of any U-shaped burrows enclosed within the chamber (Glud et al. 1995, 1996), and this factor may have operated in the *C. subterranea* deployments.

### Maxmuelleria lankesteri

Burrow openings of *Maxmuelleria lankesteri* appeared not to significantly affect sediment-water fluxes of oxygen, manganese (II), phosphate, silicate or ammonium. This was the case irrespective of whether the enclosed opening formed the ejection or the feeding end of the burrow. There is little available information on the chemical environment of echiuran burrows, but one would expect the *M. lankesteri* burrow to be hypoxic and sulphide-enriched, given its deep penetration into

anoxic, organically enriched sediments. Arp et al. (1992) found that these conditions prevailed in the burrow of *Urechis caupo*, although the ecology of this intertidal echiuran differs quite considerably from that of *M. lankesteri*. Major venting episodes involving expulsion of fluidized sediment occur on average only once every 48 h (Hughes et al. 1996), suggesting that other solutes (e.g. excretory ammonium) may also accumulate in the *M. lankesteri* burrow. The failure to detect any increased efflux of ammonium, phosphate, silicate or manganese (II) in burrow-enclosing chambers may stem from the fact that an echiuran would have been prevented from ventilating its burrow owing to the backpressure imposed by the chamber deployed over 1 opening (in contrast to the *Callianassa subterranea* experiments, in which all surface openings of each burrow were enclosed and water could be circulated within the chamber). The visual appearance of the *M. lankesteri* ejecta mounds confirmed that no sediment expulsion occurred during any of the chamber deployments. Stirring-induced flushing of the burrow should also be prevented by enclosure of 1 opening, so that the measured fluxes are likely to be purely diffusive.

In all 3 chamber groups (controls, feeding and ejection sites) most of the soluble nitrogenous material accumulated in the reduced form (ammonium). However, significantly higher effluxes of nitrate were recorded over burrow openings, particularly ejecta mounds, when compared with the 'background' sediment. This may indicate a difference in microbial populations, specifically in numbers of nitrifying bacteria, between 'background' and near-burrow sediment. If the ejecta mounds of *Maxmuelleria lankesteri* do act as localized 'hot-spots' of nitrate production, the species may indirectly play a significant role in the nitrogen cycle of its sea loch environment.

## Implications of results for future benthic chamber field studies

The results presented here show that for certain solutes, chamber fluxes overlying burrow openings of *Callianassa subterranea* and *Maxmuelleria lankesteri* could be distinguished statistically from unburrowed controls. This is true irrespective of whether the causal factor underlying the burrow effect is active ventilation, passive flushing, contrasting microbial populations, or some combination of these. Additional work will be required to determine the relative importance of these different factors. However, the ranges of variation for all measured parameters were such that it would be difficult to unambiguously identify the 'signature' of a megafaunal burrow opening in the flux data from an individual 'blind' chamber deployment

made using an autonomous lander. The ranges of variation demonstrate that sediment-water fluxes at the study site are not spatially uniform, and that megafaunal burrows contribute to this heterogeneity. Similar conclusions have recently been drawn from a biogeochemical study of the deep-sea floor in the Panama Basin (Aller et al. 1998), and the same is likely to be true for any bioturbated marine sediment.

This study includes data from a total of 45 individual benthic chamber deployments, a much larger dataset than available to most previous workers. Indeed, because of time or logistical constraints, some benthic chamber studies lack even the level of replication involved in 1 of our 5-chamber experiments. The effort expended in modelling the internal dynamics of benthic chambers under idealized conditions needs to be complemented by a greater understanding of how measured flux rates are influenced by the natural heterogeneity of the sea floor.

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