Nutrient regeneration from feces and pseudofeces of mussel Mytilus edulis spat

Nutrient regeneration from feces and pseudofeces of mussel spat (*Mytilus edulis*)

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Abstract

Suspension-feeding mussels exert top-down grazing control on primary producers, and provide bottom-up feedback of regenerated nutrients. Besides direct excretion, an important pathway of nutrient regeneration is through the decomposition of feces and pseudofeces, of which mussels can produce large quantities. Information on their quality and nutrient regeneration rates is scarce. Feces and pseudofeces, produced in varying proportions, are commonly treated as one pool. We determined nutrient regeneration rates of feces and pseudofeces decomposition in incubations using natural seawater and juvenile *Mytilus edulis* from spat collectors. Besides one 1993 trial, our results are the first to present nutrient regeneration dynamics of feces and pseudofeces separately. Dissolved inorganic nitrogen (DIN) and phosphate regeneration continued at stable rates for approximately three weeks, after which 13.1% and 12.4% of the available N and 8.7% and 7.9% of the available P was regenerated from feces and pseudofeces, respectively. Rates of silicate regeneration declined continuously, which we attribute to its accumulation in the experimental setup. Coinciding potentially limiting environmental levels of DIN and silicate indicate the potential ecological relevance of biodeposit decomposition. Overall DIN regeneration rates were similar between feces and pseudofeces, but depletion of ammonia was initially more rapid for pseudofeces due to stronger nitrification. Phosphate regeneration rates were 1.1 times greater from feces than pseudofeces, and silicate regeneration rates 1.4 times. Future research should clarify the role of bivalve suspension feeders in controlling Si and P availability in coastal ecosystems as relating to the proportion of pseudofeces generated, which depends on food concentration.

Key words

nutrient feedback, nutrient regeneration, mussel culture, mussel spat, biodeposits, feces, pseudofeces, Mytilus edulis

Introduction

Suspension-feeding mussels have a large filtration capacity, extracting important quantities of phytoplankton and other suspended matter from the water column (Cranford et al. 2011). Concurrently, metabolic losses excreted by mussels as dissolved inorganic nutrients constitute a feedback to primary producers (Prins et al. 1998, van Broekhoven et al. 2014). A second pathway of nutrient feedback is the decomposition of feces and pseudofeces (Giles & Pilditch 2006, Jansen et al. 2012b), together called biodeposits, of which mussels produce substantial quantities (Tsuchiya 1980, Smaal et al. 1986). Pseudofeces is the portion of filtered matter rejected during pre-ingestive selection, expelled in loosely mucus-bound form, and feces is the portion of ingested filtered matter egested after food processing in the digestive system (Gosling 2003). Biodeposition represents a significant pathway in bivalve nutrient cycling. For example, 40-80% of N filtered from the water can be expelled with biodeposits (Cranford et al. 2007, Jansen et al. 2012a). Biodeposits can also contain substantial amounts of P, and of silica of biogenic origin (Navarro & Thompson 1997). During biodeposit decomposition ammonia (which may then be transformed into NO_x as a result of bacterial nitrification), phosphate, and silicate are released (Giles & Pilditch 2006, Callier et al. 2009, Jansen et al. 2012b). N and P regeneration are biologically mediated, but Si primarily relies on chemical dissolution (Paasche 1980). A substantial portion of the biodeposits is decomposed within days to weeks (Giles & Pilditch 2006, Carlsson et al. 2010, Jansen et al. 2012b), so that nutrient feedback to primary producers is relevant on the short term. On average, biodeposits decompose more rapidly than phytoplankton or macroalgae (Giles & Pilditch 2006). Not all material digested by mussels is fully decomposed, with, for instance, diatoms surviving after ingestion and gut passage (Barillé & Cognie 2000).

Recently, some studies published results on mussel biodeposit decomposition (e.g. Fabiano et al. 1994, Giles & Pilditch 2006, Carlsson et al. 2010, Jansen et al. 2012b) but information on mussel biodeposit quality and nutrient regeneration rates is still scarce (McKindsey et al. 2011). So far, none of the decomposition studies have made a distinction between feces and pseudofeces decomposition patterns, which is reflected in ecosystem modelling studies (e.g. Dabrowski et al. 2013). It has been suggested that feces may decompose more rapidly than pseudofeces due to loading with bacteria from the animal's digestive system (Harris 1993, Fabiano et al. 1994). One preliminary experiment described in Smaal & Prins (1993) suggested that feces may indeed decompose more rapidly than pseudofeces, indicating the importance of studying decomposition dynamics of the two biodeposit products separately. Given the variability in the proportional contribution of pseudofeces to biodeposits in response to variability in food source and concentration (pseudofeces contribution ranging from 0-90% in Foster-Smith 1975, and a similarly large range in Tsuchiya 1980), lack of knowledge of differential nutrient regeneration rates leads to potential errors of unknown magnitude in our understanding and quantitative estimates of nutrient regeneration rates from decomposing biodeposits. The present study addresses this gap using replicated, separate incubations of feces and pseudofeces.

The study was conducted in the Oosterschelde estuary in the Netherlands, where large stocks of bivalve suspension feeders are present, possibly reaching the carrying capacity of the system (Smaal et al. 2013). In addition to the natural and cultured benthic bivalve populations, a recent development in the study area is the introduction of Seed Mussel Collector (SMC) systems (Kamermans et al. 2002). This results in additional mussel *Mytilus edulis* stocks during the summer SMC season. In this period dissolved inorganic nutrient concentrations, particularly Si and N, are

periodically at limiting levels for primary production (van Broekhoven et al. 2014). Policy decisions regarding future expansion of SMCs are informed by ecosystem model predictions of impacts on other suspension feeding bivalve populations (Meijer 2010, Kamermans et al. 2014). It has previously been shown that nutrient regeneration by bivalves can enhance primary production rates in the Oosterschelde (Prins & Smaal 1994), so that nutrient feedbacks need to be taken into account for such a model to accurately reflect the carrying capacity of the system.

The aim of the present study is twofold. Firstly, to quantify rates and proportions of nutrient regeneration from decomposing *M. edulis* spat feces and pseudofeces. The hypothesis is that measurable proportions of particulate organic nitrogen (PON), biogenic silica (BSi), and particulate organic phosphorus (POP) contained in feces and pseudofeces are regenerated within days to weeks, thereby constituting a relatively quick feedback to primary producers. Secondly, to compare release rates of N, P and Si from feces and pseudofeces. Rates are expected to be higher for feces than for pseudofeces. The study is performed under controlled conditions using replicated incubations.

Materials & Methods

Mussels

Mussels were collected from a commercial SMC in the central part of the Oosterschelde estuary (51°55'N, 3°96'E) on 5 August 2013. Shell length was determined to 0.01 mm for 387 randomly selected individuals using a digital calliper. These data were combined with length-weight (tissue plus shell) relations (R^2=0.99) established for 58 randomly selected individuals from the sample using an automatic drying (70 °C) and ashing (520 °C) apparatus (Prepash 340). Mean shell length was 15.11 (±3.52) mm, mean dry weight was 145 (±84) mg and mean ash-free dry weight was 22 (±13) mg.

Biodeposit production

Approximately 711 g (wet weight) of mussels was distributed over six cylinders of 25 cm diameter fitted with mesh bottoms (150 µm mesh size), held in a water tank (Figure 1). Unfiltered water was fed to the tank, pumped freshly from the Oosterschelde estuary at the field station (51°59'N, 3°87'E), and entering the cylinders through the mesh bottoms. Water left the setup through tubes installed at the tops of the cylinders. Water flow was regulated by visual inspection to just below the rate where biodeposit particles were occasionally observed to be transported on the upward flow and out of the setup. This way all biodeposits settled on the mesh bottoms, but supply of food to the mussels was ensured.

Suspended particulate matter (SPM) content of the water fed to the mussels was determined by averaging daily triplicate measurements on the four days leading up to and including the acclimatisation and biodeposit production periods. Samples of 1 L were transported cooled and in darkness to the laboratory for immediate filtration on Whatman GF/F filters, with salt expelled using an ammonium formiate solution. Filters were dried at 103°C for gravimetric determination as dry weight (DW) followed by combustion at 550°C for ash-free dry weight (AFDW) determination. A separate set of duplicate Whatman GF/F filters produced on the same days in the same way, but with salt expelled using demi water instead of the ammonium formate solution, was kept dry and in darkness and analysed for particulate nutrients within five months.

After placement in the biodeposit production setup, mussels were allowed to acclimatise for 48 h. The setup was cleaned daily by removing accumulated material through a small flexible tube by

force of gravity. Biodeposits produced during the following 24 h were collected through a small flexible tube in the same manner, and were used as the start material for incubations.

Feces were separated from all other material, including pseudofeces, by repeated decanting. Visual inspection confirmed the absence of feces particles from the remaining fraction. No further separation between pseudofeces and other material deposited in the production setup could be made. The contribution of non-biodeposit material to the pseudofeces fraction was estimated in a series of trials. Material deposited in a control cylinder amounted to 10% of the DW (analysis described below) and 10% of the AFDW of the total material deposited in a mussel cylinder (feces + pseudofeces + the other deposited material). In the mussel cylinder, feces made up 47% in DW and 41% in AFDW. This means that of the non-feces material, 81% of the DW and 84% of the AFDW was in fact pseudofeces. This method of biodeposit production yielded large enough quantities to enable replicated incubations in relatively large water volumes, but a limitation is that leakage of dissolved material during the production period (e.g. Carlsson et al. 2010) is not captured.

Incubations

Incubations were performed in cylindrical 520 ml transparent polypropylene containers of 95 mm height, closed with lids of the same material. To include the various constituents of the microbial loop from seawater that can contribute to nutrient regeneration rates (Azam et al. 1983, Jacobsen & Azam 1984, Poulsen & Iversen 2008) untreated seawater was selected as the incubation medium. Incubations were conducted without sediment. Regeneration from feces and pseudofeces was thereby described by the net result of decomposition and incorporation by decomposers. Three treatments were prepared: feces, pseudofeces, and untreated seawater as the control. Chambers were placed on a table in a grid pattern, with treatments assigned to chambers at random.

The incubation chambers were pre-filled with untreated seawater pumped from the Oosterschelde estuary. Biodeposits were added by transferring 30 ml from single stirred feces or pseudofeces master stock suspensions by pipette, to give a total volume of 381 ml per chamber. To determine pre-incubation composition, five 30 ml aliquots of the feces and five of the pseudofeces stocks were filtered onto Whatman GF/F filters, with salt expelled using an ammonium formiate solution. Filters were dried at 103°C for gravimetric DW determination followed by combustion at 550°C for AFDW determination. Seawater control chambers contained the total 381 ml of untreated seawater.

Chambers were gently aerated to ensure oxic conditions representing the well-oxygenated waters of the Oosterschelde estuary (Rijkswaterstaat, www.waterbase.nl), using plastic tubes fitted through the lids, in such a way that the water kept moving, but biodeposits remained settled on the chamber floors. The chambers were kept in a climate-controlled room at 20°C in continued darkness except for brief visits for sampling and checking.

Sampling of incubation chambers

Samples were analysed on days 1, 5, 7, 13, 18, 22, and 28. Incubations were continued until day 36 but most samples could not be processed due to mucous formation; this day was not analysed. On each sampling day, three replicate chambers of each treatment were selected at random and sacrificed. After uncoupling from aeration, chambers were gently swirled. From control chambers, 15 ml samples were directly transferred to 20 ml HDPE containers and stored at -18°C for total nitrogen (TN) and total phosphorus (TP) analysis within three months. Samples from day 1 were lost. Chambers were left to stand for 10 min to allow most of the particulates to settle, before further

sampling. Most of the water from the chambers was filtered through a 90 mm diameter 0.8 µm pore size cellulose acetate membrane filter (Sartorius) using a vacuum pump. Filtered water was divided into 20 ml HDPE containers and analysed within three months (Strickland & Parsons 1968, Avanzino & Kennedy 1993, Kotlash & Chessman 1998) for silicate (15 ml, stored at 4°C); total ammonia nitrogen (TAN), nitrate and nitrite, phosphate (all 15 ml, stored at -18°C), and dissolved organic nitrogen (DON, 15 ml, acidified using H_2SO_4, stored at -18°C); dissolved organic carbon (DOC, 15 ml, acidified using HCl, stored at -18°C); and dissolved organic phosphorus (DOP, 15 ml, stored at -18°C). Remaining material in chambers was subsequently mixed and acidified to pH < 3 using H_2SO_4, and subsequently transferred onto the filter by flushing with demi water, and using a spoon to remove material from chamber walls. Salt was expelled by flushing with 150 ml demi water. All particulate material was transferred from the filter to pre-weighed porcelain crucibles, followed by gravimetric DW determination (103°C) and AFDW determination (550°C). For nutrient content analysis the material from biodeposit chambers was subsequently powdered using a pestle, and samples were analysed within three months.

Nutrient analysis

Amounts and concentrations of Si, N or P containing compounds are quantified in terms of their constituent element Si, N, or P. Silicate concentrations were determined by Seal QuAAtro segmented flow analyser (Jodo et al. 1992, Aminot et al. 2009). Ammonia, phosphate, and NO_x (determined as nitrate plus nitrite), were determined using a Skalar San++ segmented flow analyser (Aminot et al. 2009). Dissolved inorganic nitrogen (DIN) was calculated as the sum of ammonia and NO_x. Total dissolved nitrogen (TDN) was determined from filtered water samples as NO_x following persulfate and subsequent UV digestion (Kroon 1993, Eaton et al. 1999), and dissolved organic nitrogen (DON) was determined by subtraction of DIN. Total nitrogen (TN) and total phosphorus (TP) were determined as NO_x and phosphate after 30 min oxidation at 120°C in stopped volumetric flasks each containing 25 ml of unfiltered water sample and reagent (50 g L^{-1} potassium peroxodisulfate, 7.5 g L^{-1} sodium hydroxide), and filled up to 100 ml (Valderrama 1981). Dissolved organic phosphorus (DOP) was determined using the same procedure applied to filtered water samples, and by subtraction of phosphate. Dissolved organic carbon (DOC) was determined from filtered water samples using a Skalar San++ segmented flow analyser using photochemical conversion to CO_2 and infrared detection (e.g. Collins et al. 1977; according to NEN-EN 1484). Particulate organic N (PON) and P (POP) from incubation chambers were determined using a Skalar San++ segmented flow analyser after digestion using a H_2SO_4/Se/salicylic acid/H_2O_2 solution (Temminghoff & Houba 2004). Biogenic Si (BSi) was determined after a 3 h extraction of 25 mg of feces in 25 mL of 0.5M NaOH solution and subsequent analysis of extracted silicate concentration on a Skalar San++ segmented flow analyser (adapted after DeMaster 1981, see Barão et al. 2015). Particulate organic C (POC) from incubation chambers was determined using a Thermo-spectronic Aquamate spectrophotometer following oxidation at 135°C in a solution of H_2SO_4 and $K_2Cr_2O_7$ (Walinga et al. 1992). C and N content of the material collected on filters from the water supply were freeze-dried and ground to a fine powder for analysis on a Interscience Flash 2000 organic element analyser (Nieuwenhuize et al. 1994). P content of this material was analysed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, ThermoFisher iCAP6500) after digestion with HNO_3 at 200°C using Ytterbium as internal reference standard (Poussel et al. 1993).

Calculations & statistics

Throughout the text error values and error bars represent standard deviations unless otherwise specified. The term nutrient "release" is used in this study to refer to the net balance of underlying nutrient uptake and release processes, where the balance represents accumulation of a nutrient.

PON and POP were determined as N and P content of particulate dry mass. Amounts of ammonia, NO_x, DON, phosphate, DOP, DOC, and silicate in chambers were normalised to one gram of feces or pseudofeces. Concentrations were multiplied by the water volume in the chamber, and divided by the dry particulate mass in the chamber, which was calculated as particulate plus dissolved matter present on the sampling day minus mean dissolved matter in chambers sampled on day 1. This accounts for the loss of water due to aeration, amounting to 2.3 ml d^{-1} on average. This method assumes that the total amount of material (particulate plus dissolved) did not change throughout the experiment. Linear regression analysis of the mass of total material over time confirmed that there was no significant change in the total material throughout the experimental period. To allow comparison, control concentrations (after multiplication by the water volume in the chamber) were standardised by scaling fluxes using the average dry particulate mass used to standardise the experimental chambers to 1 g biodeposit (0.5175 g).

Trends in nitrogen and phosphorus variables were calculated after subtraction of controls and tested using linear regression through the origin for the first 18 days, marking the cessation of accumulation of DIN, which was followed by decreasing concentrations indicating a switch to predominant removal of nutrients. Accumulation of phosphate ceased later, and accumulation of silicate did not cease at all, but all parameters were analysed over the same period for comparability. A coinciding formation of increasing quantities of slimy material observable on chamber walls after the period of accumulation might be an artefact of the experimental setup or the use of incubation vessels; the analyses do not include this period. Trends in silicate were tested using power regression since chemical dissolution leading to increasing concentrations of silicate is expected to lead to diminishing release rates (e.g. Struyf et al. 2007). Regressions were considered significant when $p<0.05$. Power regressions for DIN and phosphate did not indicate suppression of accumulation by approaching equilibrium. Hence, the linear regressions were used for further calculations. Release rates were compared between biodeposit types and between nutrient parameters per biodeposit type using analysis of covariance with either biodeposit type or nutrient parameter as the categorical variable, with results considered significant when $p<0.05$. Initial release rates were calculated by derivative from regression equations, and expressed as daily release in per cent of the particulate amount present on day 1. Stoichiometric ratios of N:P, N:Si and Si:P were calculated on a molar basis. Ratios in released nutrients were based on the derivative from regression equations on day 1.

Results

Initial conditions

On the four days leading up to and including the day of biodeposit production the water supply feeding the mussels contained 9.6±1.4 mg L^{-1} SPM (DW), of which 37.5% was organic matter. In the SPM, 0.66±0.10 mg C L^{-1}, 0.06±0.01 mg N L^{-1}, and 0.014±0.002 mg P L^{-1} was present.

Slightly more dry mass of pseudofeces was added to chambers than of feces at the start of incubations (not intentional; Table 1). Pseudofeces contained more organic matter, C and N than

212 feces, and feces contained more Si than pseudofeces, whereas P content was similar in both
213 compartments. Control chambers contained 1.0±0.8 mg dry mass of particulate material on day 1.

214 Dissolved nutrients

215 DIN and phosphate declined after day 22 and 18, respectively. During the first 18 days, the average
216 DIN release rates from feces and pseudofeces after subtraction of controls were 0.035 and 0.037 mg
217 g^{-1} DW d^{-1}, respectively (Table 2), which was not significantly different. Expressed per unit initial N,
218 0.73 and 0.69% d^{-1} were regenerated from feces and pseudofeces, respectively. During the same
219 period, the phosphate release rate from feces after subtraction of controls was significantly higher
220 (0.007 mg g^{-1} DW d^{-1}) than from pseudofeces (0.006 mg g^{-1} DW d^{-1}). Expressed per unit initial P, 0.48
221 and 0.44% d^{-1} were regenerated from feces and pseudofeces, respectively (Table 2). The elemental
222 release rate of N was significantly greater than that of P, and the fraction of initial feces and
223 pseudofeces N released daily was approximately twice that of P. The first 18 days represented the
224 release of 13.1% and 12.4% of initial N in feces and pseudofeces, respectively, and 8.7% and 7.9%,
225 respectively, of initial P.

226 In the first week, ammonia accumulated in feces chambers, while in pseudofeces chambers
227 there was a much lower accumulation which peaked on day 5 (Figure 3). The accumulation in feces
228 chambers was similar to that in the controls. At the same time, NO_x was released faster in
229 pseudofeces chambers than in feces chambers at the start of the study, and appeared to accelerate
230 slightly after the first week (Figure 3). In feces chambers NO_x release started more slowly and
231 accelerated more after the first week, catching up with pseudofeces chambers after three weeks.

232 Silicate release followed power functions throughout the incubation period, with release
233 rates diminishing progressively (Figure 2; Table 2). There was a very small but significant decrease of
234 silicate in the controls. Release rates after subtraction of controls were significantly greater from
235 feces than from pseudofeces, with 1.21% d^{-1} regenerated on day 1 from feces and 0.84% d^{-1} from
236 pseudofeces. The first 18 days represented the release of 11.0% (feces) and 6.8% (pseudofeces) of
237 initial BSi.

238 Concentrations of DON and DOP were low compared to their dissolved inorganic forms, and
239 concentrations in controls were similar to feces and pseudofeces chambers. There were significant
240 trends of reduction of DOP and DOC in pseudofeces during the first 18 days, but there were no
241 trends in DON, feces, or controls (Figure 4). After subtraction of controls, a statistically significant
242 reduction of DON was found, in feces, which was small compared to the DIN accumulation (DON:
243 0.05% d^{-1}; DIN: 0.73% d^{-1}). Similarly, a statistically significant reduction of DOP was found, in
244 pseudofeces, which was small compared to the phosphate accumulation (DOP: -0.03% d^{-1};
245 phosphate: 0.44% d^{-1}). DOC could not be calculated in this way as it was not measured separately in
246 controls. After day 18 there was a sudden increase in DOP in all treatments, which can also be
247 observed in DOC to a lesser extent.

248 Particulates and nutrient balance

249 Near the end of the incubation period increasing amounts of mucous material were observed in
250 biodeposit chambers, and filtration on the 90 mm ø, 0.8 µm pore size membrane filters became
251 increasingly difficult. However, there were no significant trends in recovered dry mass of total
252 particulate material over the incubation period.

253 There were no significant trends in PON for feces and pseudofeces, and for POP there was
254 only a trend in feces (Figure 5), of -0.67% d^{-1} after subtraction of controls. The total amounts of N and

P per chamber over the incubation period were examined for trends over the first 18 days. Dissolved inorganic, dissolved organic, and particulate organic constituents were summed to estimate total amounts of N and P on each sampling day. After subtraction of controls, the summed N in feces pseudofeces chambers showed a significant increase over time of 0.61% d^{-1}, which was not significantly different from the increase of DIN (Table 2; 0.73% d^{-1}). There was no trend in the controls. The summed P did not show any significant trends.

Nutrient stoichiometry

The difference between feces and pseudofeces in N:P ratio of regenerated nutrients was limited, whereas feces released proportionally more Si than pseudofeces (Table 3).

Biodeposit decomposition influenced the stoichiometry of dissolved inorganic nutrients in the surrounding water. The N:P ratio of dissolved inorganic regenerated nutrients was lower than Redfield's ratio, but was higher than that of the particulate material on day 1, which in turn was more than double that of dissolved inorganic nutrients in the Oosterschelde water. Availability of N was thus promoted relative to P. Additionally, a considerable surplus of Si relative to N and P in the regenerated nutrients was evident, since N:Si was lower, and Si:P was considerably higher, than both the Oosterschelde water and Redfield's ratio.

Discussion

Mineralisation rates

Processes

The rates as determined in this study represent the net balance of underlying processes. During organic matter decomposition, nutrients are released to the environment in various forms contributing to the dissolved inorganic or organic nutrients, while another part is incorporated by bacteria (Horrigan et al. 1988, Canfield et al. 2005) or by other constituents of the heterotrophic food web (Azam et al. 1983, Fabiano et al. 1994). During the incubations, the period of linear accumulation of DIN and phosphate was followed by decreasing concentrations after days 22 and 18, respectively, indicating a switch to predominant removal of nutrients. This coincided with formation of increasing quantities of slimy material, a mix of microorganisms and trapped organic matter, on chamber walls. This might be an artefact of the experimental setup or the use of incubation vessels, and therefore calculations and comparisons are only based on the period up to this point.

The balance of regeneration and incorporation depends on the proportional nutrient composition of the substrate (Goldman et al. 1987, Tezuka 1990, Canfield et al. 2005). The nutrient composition of the biodeposits in the present study (feces C:N 12.7; pseudofeces C:N 11.9; feces N:P 7.6, pseudofeces N:P 8.4) suggests that the amount of regeneration may be expected to be low and accompanied by high levels of incorporation. C:N content was not much higher than reported for natural marine bacterial assemblages by Goldman et al. (1987), who found little or no ammonia release during the exponential growth phase at a low C:N value of 10. These authors reported that some ammonia release did occur during the subsequent stationary phase when endogenous metabolism and cell death became dominant processes. Tezuka (1990) described interactions between substrate C:N and N:P ratios for freshwater bacterial communities, observing that both ammonium and phosphate were regenerated when both N and P content were high enough (C:N ≤ 10 and N:P ≤ 16), but that neither was regenerated when N and P content were at low levels (C:N ≥

15 and N:P ≥ 5). Feces and pseudofeces in the present study lay between these combinations. Several factors may modify these relationships in the context of the present study. Firstly, the nutrient ratios of the complex substrates investigated in the present study may not necessarily correspond to nutrient ratios of the portion of the substrate actually undergoing decomposition (Tezuka 1989, 1990, Canfield et al. 2005). Secondly, the involvement of other organisms present in the untreated seawater used in the current study, for instance primary and secondary consumers of bacteria such as flagellates and microzooplankton (Azam et al. 1983, Jacobsen & Azam 1984), or dinoflagellates (Poulsen et al. 2011), may influence regeneration rates and dynamics. Finally, in making the translation to natural situations where sunlight is available to support primary production, the share of nutrients captured by heterotrophic microbes may be reduced due to competition with primary producers (e.g. Fuhrman et al. 1988, Danovaro 1998), which would effectively increase the efficiency of the nutrient feedback as more nutrients might be available for primary producers than expected based on the outcomes of the current study.

Dissolved inorganic nutrients

DIN and phosphate accumulated until sampling days 22 and 18, respectively. A comparison can be made in terms of the overall fraction of organic start material which is regenerated into the dissolved inorganic phase in the first 18 days. Values were compared to literature describing biodeposits produced by adult mussels; the use of mussel spat in the present study should be kept in mind. The 12-13% of initial PON released as DIN during this period was lower than results reported by Jansen et al. (2012b), who created stable state conditions analogous to a bioreactor by adding fresh biodeposits daily, and found that overall 17% of PON was released to the environment as ammonia. A similar value of 18% release of PON as ammonia was estimated by Giles & Pilditch (2006) for biodeposit decomposition on sediment cores over 10 days. Another perspective is provided by comparing rates of nutrient regeneration, which shows that daily release of DIN expressed as per cent of initial PON (feces: 0.73% d^{-1}; pseudofeces: 0.69% d^{-1}) was less than half of values reported by Giles & Pilditch (1.8% per day; 2006).

Smaal & Prins (1993) estimated very high rates of 4.6% d^{-1} PON regeneration for feces, and 1.6% d^{-1} for pseudofeces. In further contrast, Fabiano et al. (1994) reported 87% decomposition of organic matter from mussel fecal material within 3 d; part of this material constituted inorganic nutrient regeneration. However, proportional nutrient regeneration rates could not be compared as quantitative information regarding the start material was not provided by these authors.

A comparison as described above for PON could not be carried out for POP, since we did not find studies in literature combining reliable estimates for initial POP content of bivalve biodeposits with release rates of phosphate. Similarly, no studies were found giving initial BSi content. However, previous research can be compared in terms of stoichiometric proportions of released nutrients. N:P ratios (feces 17, pseudofeces 15) were within the range reported in literature, being higher than the range given by Jansen et al. (2-12; 2012b), but lower than reported for benthic biodeposit decomposition by Callier et al. (27; 2009) and reported for sediment core biodeposit decomposition by Giles & Pilditch (elevated by 27; 2006). Si:P ratios (feces 84, pseudofeces 45) were within the range reported by Jansen et al. (2-143; 2012b), and higher than reported by Callier et al. (36; 2009). N:Si ratios (feces 0.2, pseudofeces 0.4) were within the range given by Jansen et al. (0.0-1.3; 2012b), but this was lower than the value reported by Callier et al. (0.7; 2009). This indicates that relatively more P and Si regeneration was observed in the present study than reported by Callier et al. (2009), and relatively more P than reported by Giles & Pilditch (2006).

The studies by Jansen et al. (2012b) and Giles & Pilditch (2006) both reported that DIN release was dominated by ammonia, whereas in the present study NO_x was the dominant form. Giles & Pilditch (2006) hypothesised that coupled nitrification-denitrification could have removed ammonia, and this might account for part of an additional estimated 34% PON regeneration that was not detected as ammonia. We do not expect meaningful levels of denitrification and N_2 production in the present study since a well oxygenated system was applied. However, nitrification was likely an important process, as accumulation of TAN in feces and control treatments early on during incubations was followed by its removal and concurrently rising NO_x concentrations. This likely reflects the development of a nitrifying microbial community reaching considerable nitrifying capacity only after several days. Ammonia accumulated initially since the oxidation of this compound is considered to be the rate-limiting step in nitrification (Kaplan 1983, Canfield et al. 2005). In the pseudofeces treatment, a shorter period and lower levels of ammonia accumulation, and more rapid NO_x accumulation, point to a more rapid establishment of nitrifying capacity, suggesting that the associated microbes were more present or more active in pseudofeces than in feces and in (control) seawater.

Regeneration of BSi differs fundamentally from PON and POP because it relies on chemical dissolution rather than biological processes (Paasche 1980). The diminishing rate of Si accumulation over time, with accumulation following a power law, could be indicative of the substrate becoming less degradable during chemical dissolution. However, earlier experiments with dissolved Si release from litter of *Phragmites australis* (common reed), indicated that rather than the substrate becoming less degradable, dissolution is impacted by decreasing rates due to accumulation of dissolved material, and an equilibrium concentration is reached (Struyf et al. 2007). In our experiment, dissolved silicate concentrations at the end of the experiment on day 28 were 475.8±35.9 µmol L^{-1} for feces and 325.1±17.5 µmol L^{-1} for pseudofeces, which is not as high as equilibrium concentrations reached during the reed decomposition experiment (approximately 1500 µmol L^{-1}), but the power function still indicates a reducing release rate during the experiment. Solid (as total amount of incubated BSi)-solution rates in the beginning of our experiment were about one order of magnitude lower than in the experiment described in Struyf et al. (2007), and initial BSi content in the litter of reed (6% BSi) was higher compared to feces (4,2% BSi) and pseudofeces (3.1% BSi). This could explain why saturation was attained in the reed experiment after 30 days, but not yet in our experiment. Due to the different initial conditions, a quantitative comparison of the release rates between both experiments is difficult. At the end of the experiment, 22% of the incubated BSi had been dissolved from the feces, and 17% had been dissolved from the pseudofeces. This indicates that not only did feces contain relatively more BSi compared to pseudofeces; feces released a larger part of the BSi over the same time period, emphasising that the initial BSi content alone cannot explain the faster release from the feces.

The relative importance of Si release relative to N and P was likely underestimated for two reasons. Firstly, since silicate concentrations were higher than encountered environmentally and thus suppression of BSi release was elevated. Concentrations of silicate on day 1 (feces chambers: 1.06 mg L^{-1}; pseudofeces: 0.78 mg L^{-1}) were already elevated compared to maximum environmental concentrations during the main SMC season in 2013 of June-August (0.25 mg L^{-1}). Secondly, since release rates were calculated for day 1 rather than approaching the very start of the incubations in order to avoid extrapolation outside of the measured range.

Nutrient balance

The low concentrations and stability or relatively small absolute changes of dissolved organic matter concentrations throughout the incubation period suggest that either limited production of dissolved organic matter occurred during incubations, or that production was matched by loss due to processing rates. Since biodeposit production took place over a 24 h period, loss of labile material may have occurred before the start of incubations, with concentrations in chambers having stabilised before measurements started. This may have led to an underestimation of overall nutrient regeneration. In literature, rapid leakage of dissolved organic matter from feces has been argued to occur in the first hours after production. Carlsson (2010) estimated that 2% POC h^{-1} was lost from mussel fecal pellets during the first 24 hours, a large part of which was not regenerated, and speculated that this part of removal represented leakage of DOC. But it is also possible that this part consisted of particles, microbes or other larger compounds (e.g. Jacobsen & Azam 1984). Fabiano et al. (1994) reported that mussel fecal material decomposed into dissolved inorganic nutrients with little change in dissolved organic matter concentrations. Møller (2003) reported DOC leakage rates from copepod fecal pellets exceeding 20% of POC within the first hour after production, with the rate of leakage already rapidly levelling off during this period. Using the estimate of Carlsson et al. (2010), under the assumption that feces and pseudofeces lose POC at a similar rate, approximately 21% of POC could have been lost during the production setup.

Along with the release of dissolved inorganic N and P, and considering that there was little change in the dissolved organic phase, a reduction of PON and POP is expected. However, a reduction was only detected for POP, and only for feces. The rate of decrease of 0.67% d^{-1} was not significantly different from the negative of the rate of increase in phosphate of 0.48% d^{-1}. Slimy material formed on chamber walls throughout the experiment, but it cannot be verified whether inconsistent completeness of recovery of this material could have contributed to variability and hindered detection of reduction of PON. The sum totals of N and of P were expected to remain constant during incubations. In the case of P this was verified, but the sum total of N increased for both feces and pseudofeces. We expect the analytical methodology to be robust to structural and chemical changes occurring in particulate material over the course of the incubations. However, concentrations were relatively low, increasing the likelihood of not detecting changes. Further research is needed to verify N dynamics in the different compartments and should focus on the methodology.

Comparison of feces and pseudofeces

In contrast to the proposition that bacteria contained in feces may accelerate mineralisation (Gowing & Silver 1983, Harris 1993, Fabiano et al. 1994), our results showed similar N regeneration per unit mass from feces and pseudofeces, despite different dynamics of ammonia and NO_x, and a higher release rate of P regeneration from pseudofeces. It is possible that bacterial colonisation after egestion, which can be very rapid (Stuart et al. 1982, Jacobsen & Azam 1984), and which could potentially be more so due to greater surface:volume ratios in biodeposits produced by juvenile mussels, may have overshadowed any “head start” of the feces. Possibly, the bacterial community promoted by the bivalve enteric environment (e.g. denitrifiers, Stief et al. 2009, Svenningsen et al. 2012) does not perform very effectively in an oxic environment after egestion. In fact, it appears that pseudofeces experienced a “head start” with regard to nitrification, with the formation of nitrifying capacity requiring a shorter lag phase than for feces. It should be noted that nutrient regeneration rates from pseudofeces should be interpreted as an approximation since part of the material was

natural sedimented material that was deposited in the production setup; this also resulted in slight organic enrichment of the pseudofeces material (the sedimented material contributed 23% to pseudofeces DW but 28% to AFDW).

Si dissolution rates were higher for feces per unit initial biodeposit DW, and 1.4 times higher per unit initial BSi. We here hypothesise that two processes cause the difference between feces and pseudofeces. Firstly, we suggest that the organic matrix surrounding the BSi is broken down more strongly in the feces, which is reflected in the higher dissolution rate. A similar observation has been found in cattle, where grass BSi dissolved much quicker after digestion, as digestion removed the organic matrix surrounding the BSi (Vandevenne et al. 2013). In cattle feces, a stronger digestion of organic matrices can be expected compared to pseudofeces, which would explain the stronger dissolution. Bidle & Azam (1999) also observed that bacterial activity can accelerate silica dissolution by breaking down the organic matrix protecting diatom frustules. In the *P. australis* decomposition experiment by Struyf et al. (2007), suppression of bacterial activity also slightly decreased Si release rates. Secondly, Dame et al. (1991) speculated that dissolution rates of diatom frustules can be accelerated by fragmentation during digestion. If diatoms are more fragmented in feces then pseudofeces, this could further explain the difference in dissolution rates. Given the rising proportion of pseudofeces with increasing food concentration beyond a certain level (Foster-Smith 1975, Tsuchiya 1980), the role of bivalve suspension feeders in terms of Si regeneration could be relatively greater at lower food concentrations, assuming food composition does not change. As an alternative hypothesis, mussels could also potentially actively select for ingestion of least recalcitrant BSi, causing increased solubility of feces BSi. Biogenic Si can differ in solubility due to several factors, including specific surface and aluminum content (Van Cappellen et al. 2002).

Nutrient feedback and limitation

During the SMC growth seasons of 2012 and 2013, N and Si concentrations, but not P concentrations, in the study area were at times below the half-saturation coefficient for phytoplankton uptake (N and Si: 2 µmol L^{-1}; P: 0.2 µmol L^{-1}) (Rijkswaterstaat, www.waterbase.nl), suggesting that N and/or Si, and not P, availability was likely limiting primary production at those times (Philippart et al. 2007, Kromkamp et al. 2013, for discussion on limiting nutrients see also van Broekhoven et al. 2014). In the present study N and Si were released during biodeposit decomposition, and this lead to N and Si enrichment relative to P. Furthermore, Si was regenerated at a faster relative rate than N. In a context of N and Si limitation, regeneration of nutrients through decomposition of mussel biodeposits thus has the potential to stimulate primary productivity.

Wikfors (2011) argued that entrapment of diatom frustules in biodeposits might promote a non-diatom algal community – potentially containing harmful species – through preferential N and P recycling relative to Si. Our research, however, suggests that SMCs actually contribute to reduction of Si limitation through preferential recycling of Si compared to N and P, and thus has the potential to stimulate growth of diatoms. In the Bay of Brest, recycling of BSi by the invasive suspension feeder *Crepidula fornicata* was considered an important factor for the avoidance of harmful algal blooms in summer (Ragueneau et al. 2002).

Conclusions

Substantial regeneration of N, P and Si from decomposing mussel biodeposits was measured. There was no significant difference between feces and pseudofeces in terms of overall DIN (feces: 0.73% d^{-1}; pseudofeces: 0.69% d^{-1}) regeneration rates, but early DIN dynamics were different in terms of

more rapid depletion of ammonia due to nitrification in pseudofeces. Regeneration rates of phosphate were 1.11 times higher from feces (0.48% d^{-1}) than from pseudofeces (0.44% d^{-1}). Silicate regeneration rates were 1.43 times higher from feces (1.21% d^{-1}) than from pseudofeces (0.84% d^{-1}). During the summer season when SMCs are deployed, shortages of N and Si in the study system, the Oosterschelde, indicate that nutrient regeneration from biodeposit decomposition constitutes an important feedback pathway that needs to be quantified in order to assess aquaculture impacts. Our results add to the growing evidence that, besides having the capacity to control N and P circulation in ecosystems, producer-consumer interactions can also play an important role in the regulation of the global Si cycle.

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646

647

Figure 1

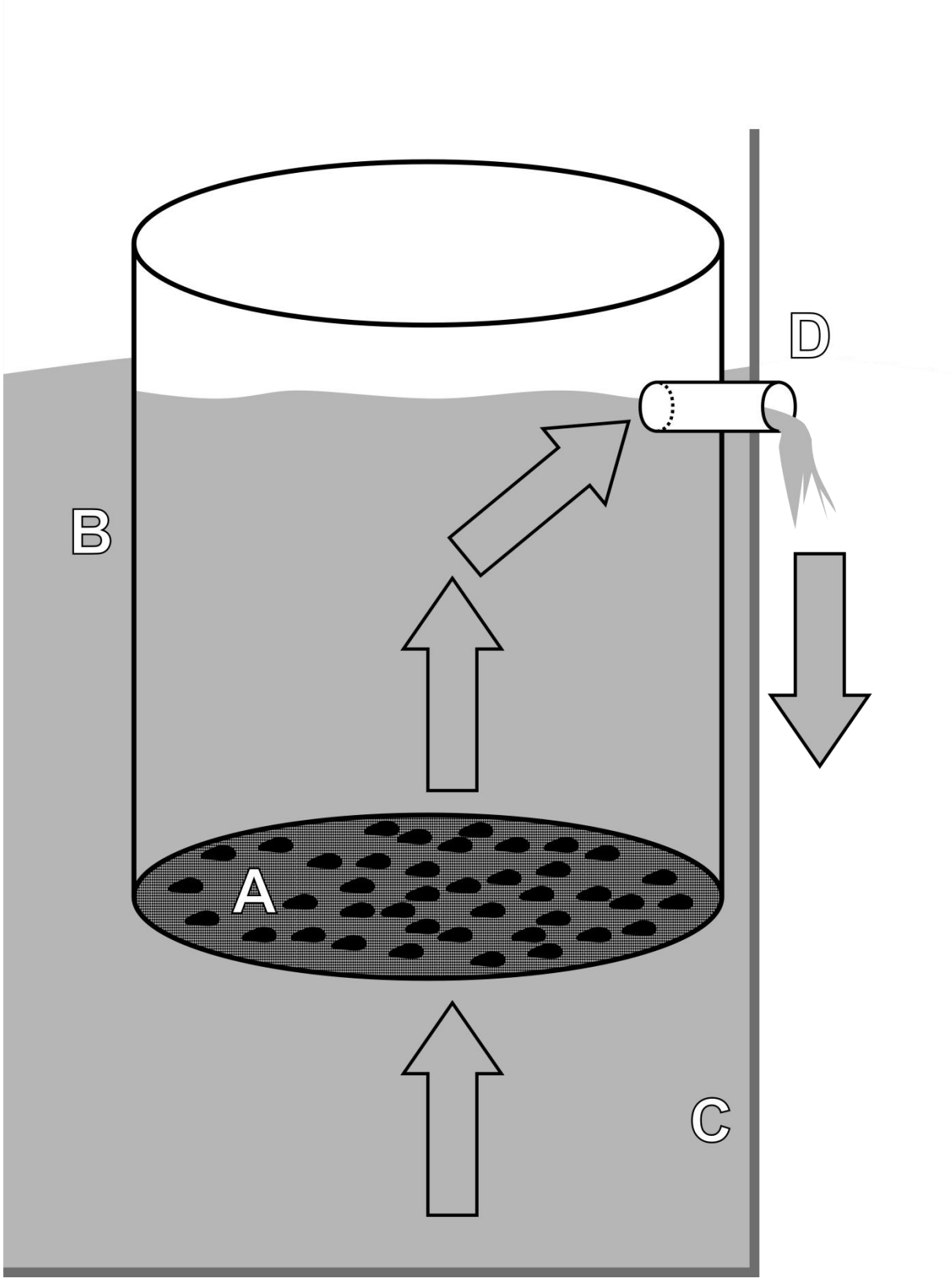
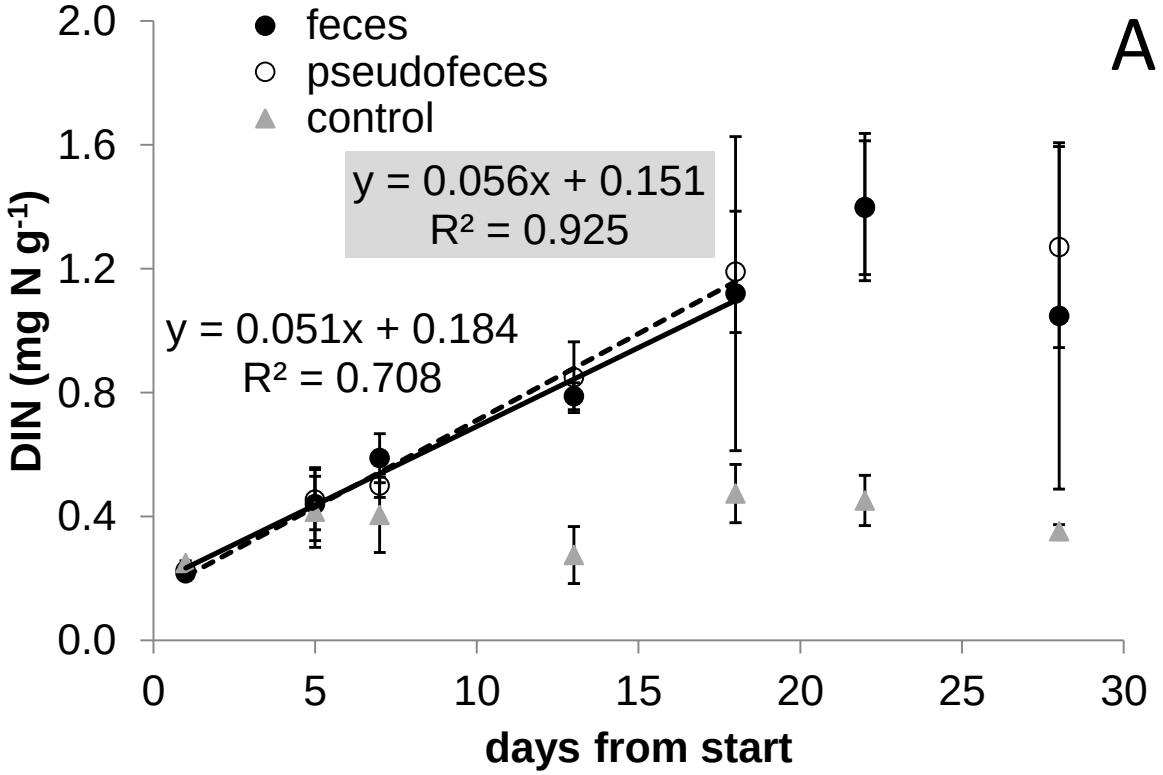
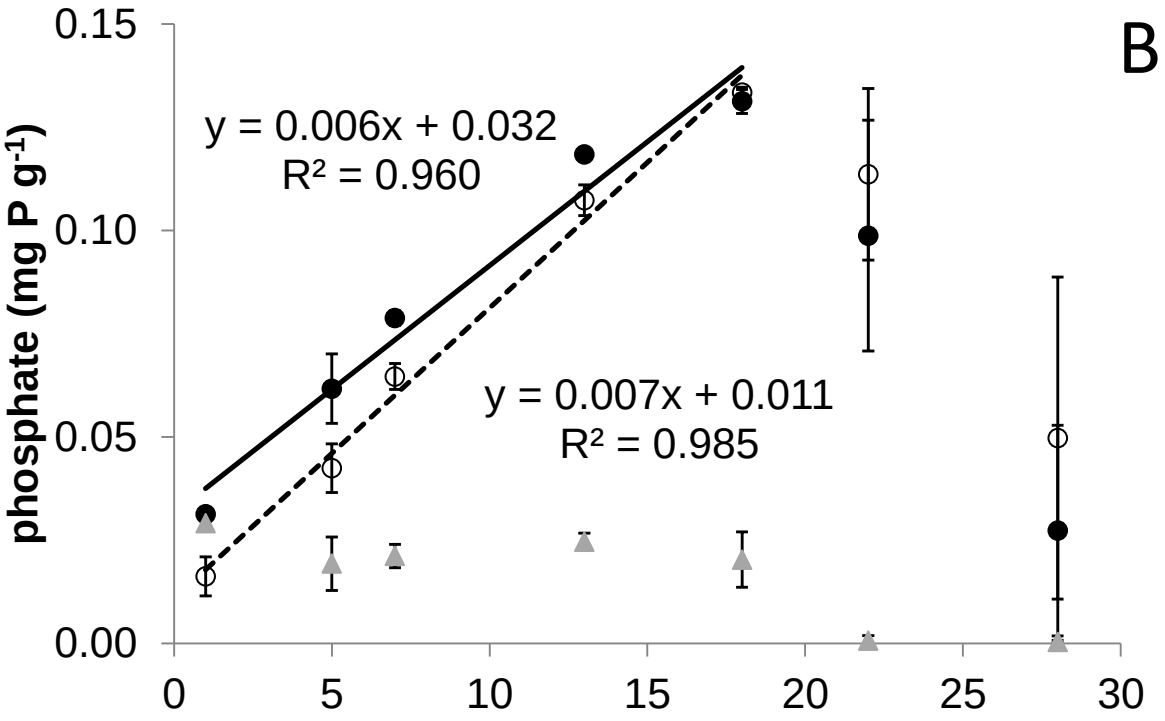


Figure 1. Schematic of part of biodeposit production setup (not to scale); side view of one of the six cylinders showing tank section. A: mesh with mussels and accumulating biodeposits; B: cylinder; C: tank wall; D: water exit tube, penetrating tank wall and tightly fit. Water is pumped into the tank; arrows indicate direction of water flow.

Figure 2


A
feces
pseudofeces
control
y = 0.056x + 0.151
R² = 0.925
y = 0.051x + 0.184
R² = 0.708
DIN (mg N g-1)
2.0
1.6
1.2
0.8
0.4
0.0
0
5
10
15
20
25
30
days from start


B
y = 0.006x + 0.032
R² = 0.960
y = 0.007x + 0.011
R² = 0.985
phosphate (mg P g-1)
0.15
0.10
0.05
0.00
0
5
10
15
20
25
30

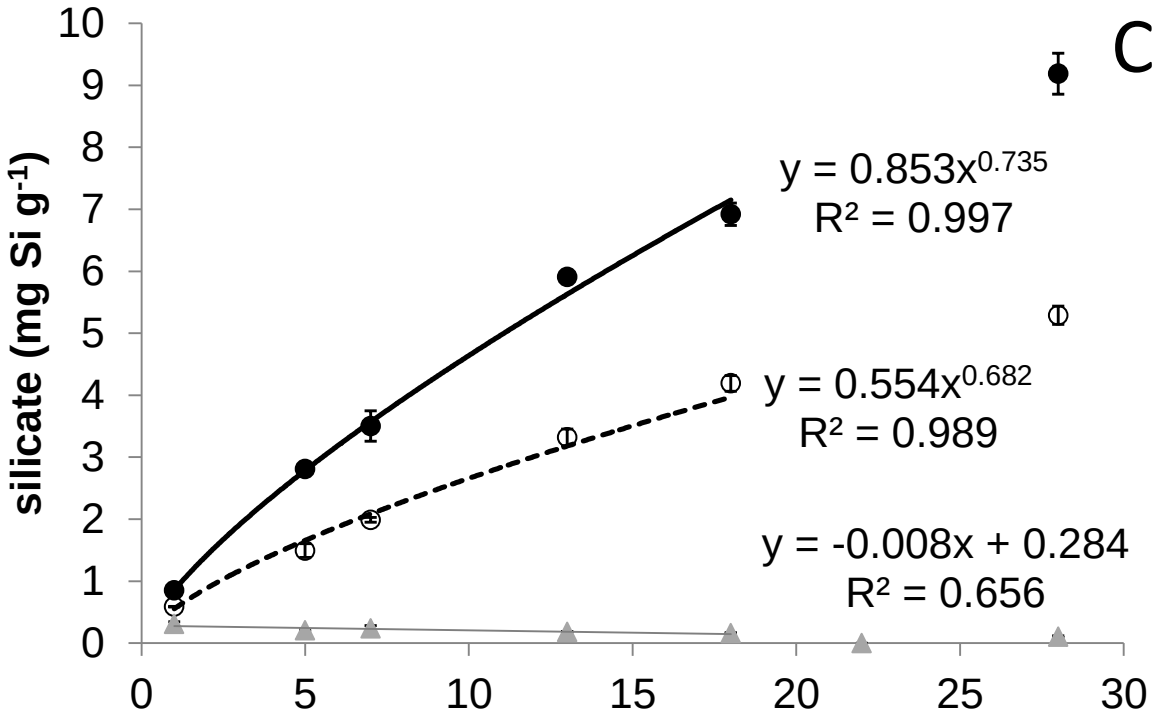
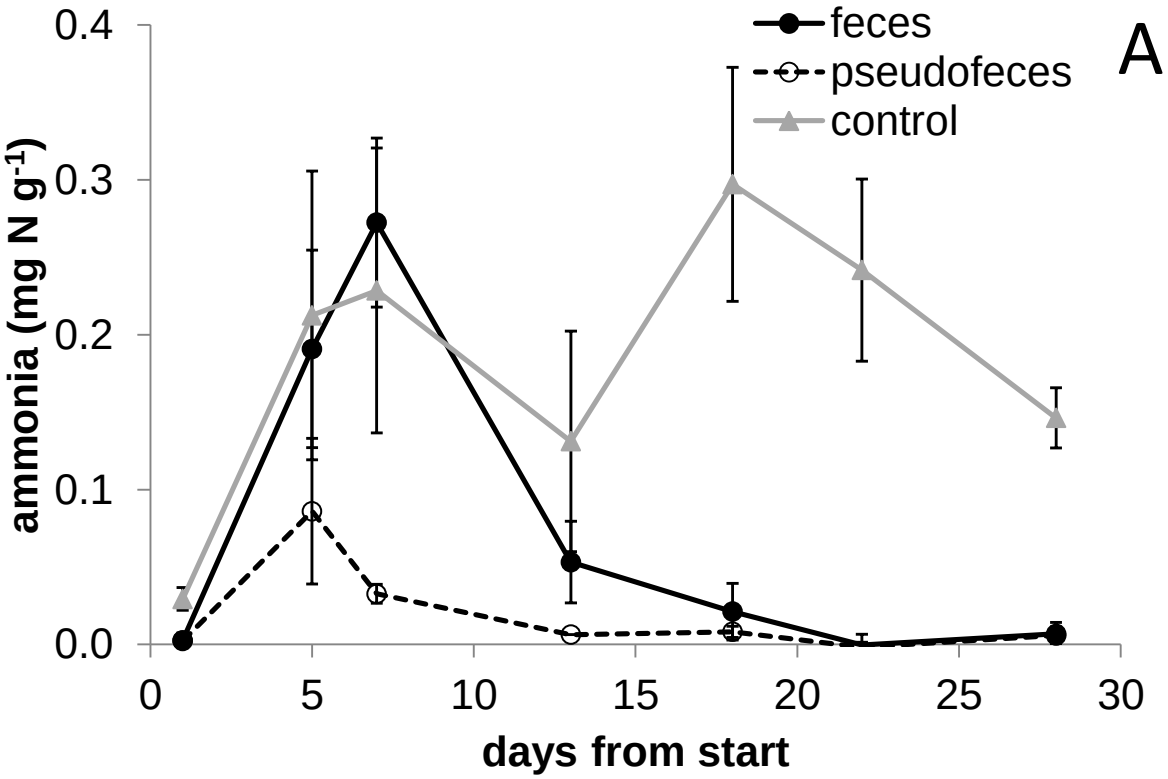


Figure 2. Dissolved inorganic nutrient quantities throughout the incubation period (element mass standardised to dry mass of start material in chamber; error bars indicate SD; n=3 for each treatment). Panel A: DIN; B: phosphate; C: silicate. Lines (unbroken: feces; broken: pseudofeces; grey: control) represent all significant regressions over the first 18 days (equations and R^2 shaded for pseudofeces in panel A); linear for DIN and phosphate and power for silicate.

Figure 3


feces
pseudofeces
control
A
ammonia (mg N g-1)
0.4
0.3
0.2
0.1
0.0
0
5
10
15
20
25
30
days from start

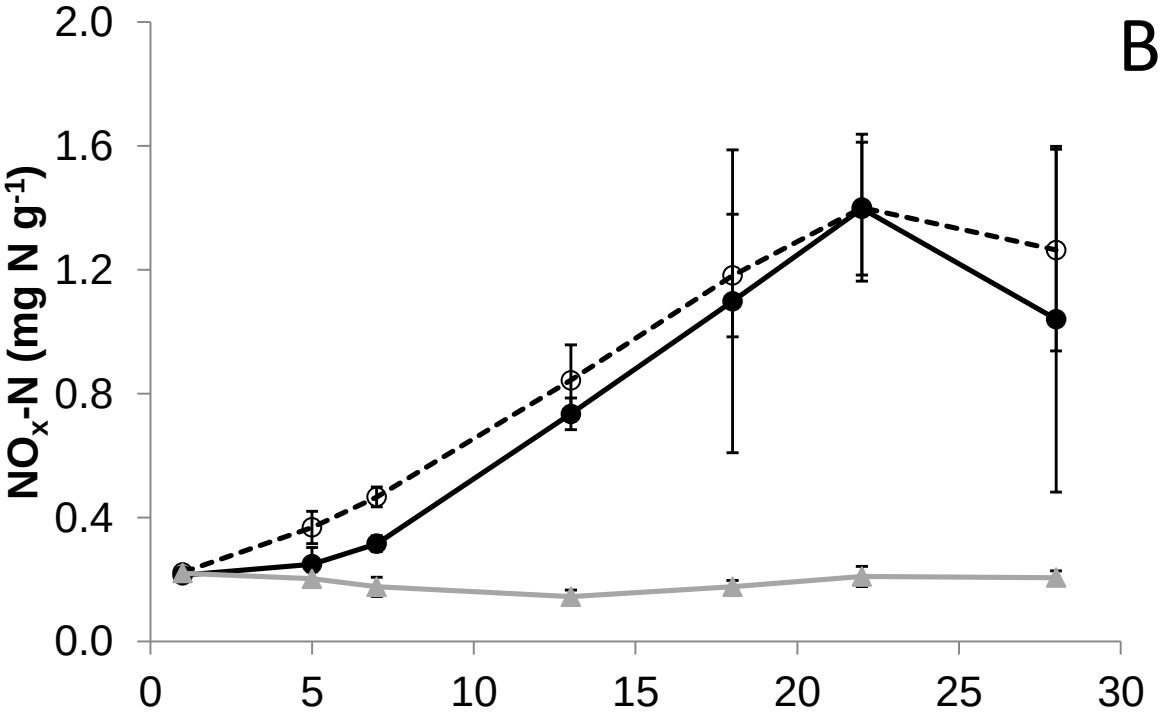
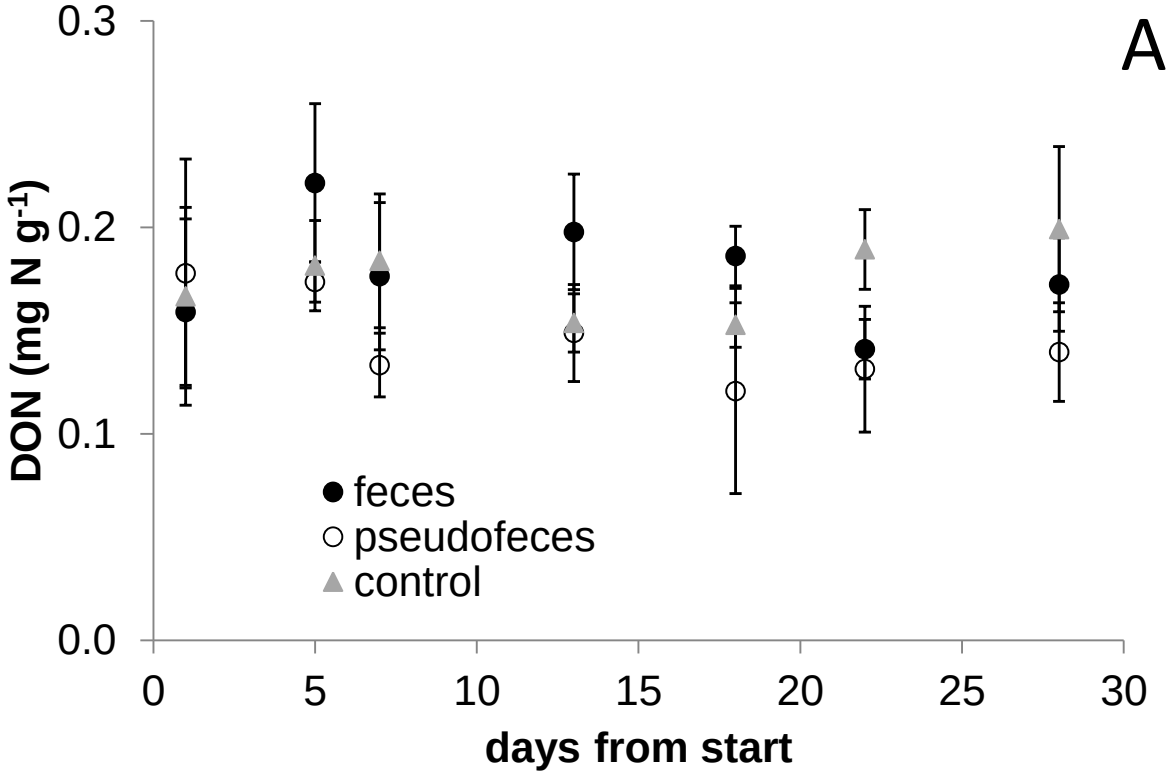
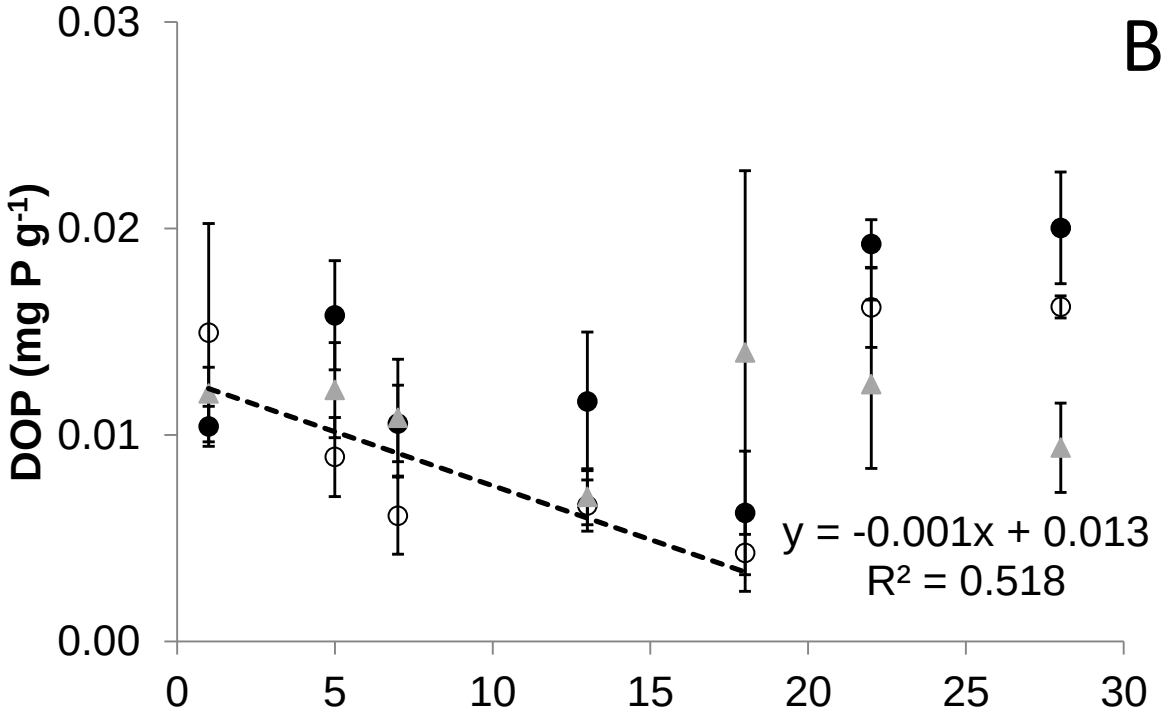


Figure 3. Detail of the constituent parts of DIN: quantities of ammonia (panel A) and NO_x (panel B) during the incubation period (element mass standardised to dry mass of start material in chamber; error bars indicate SD; n=3 for each treatment). Data points are connected by straight lines.

Figure 4


A
0.3
0.2
0.1
0.0
DON (mg N g-1)
feces
pseudofeces
control
0
5
10
15
20
25
30
days from start


B
DOP (mg P g-1)
0.03
0.02
0.01
0.00
0
5
10
15
20
25
30
y = -0.001x + 0.013
R² = 0.518

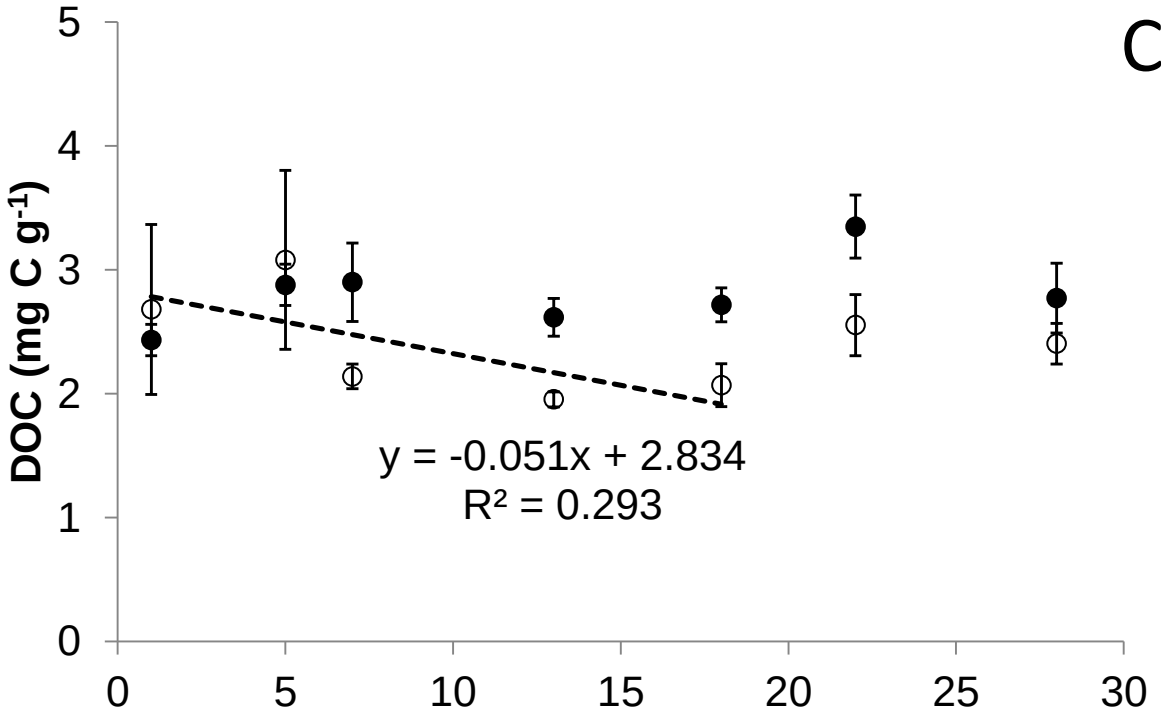
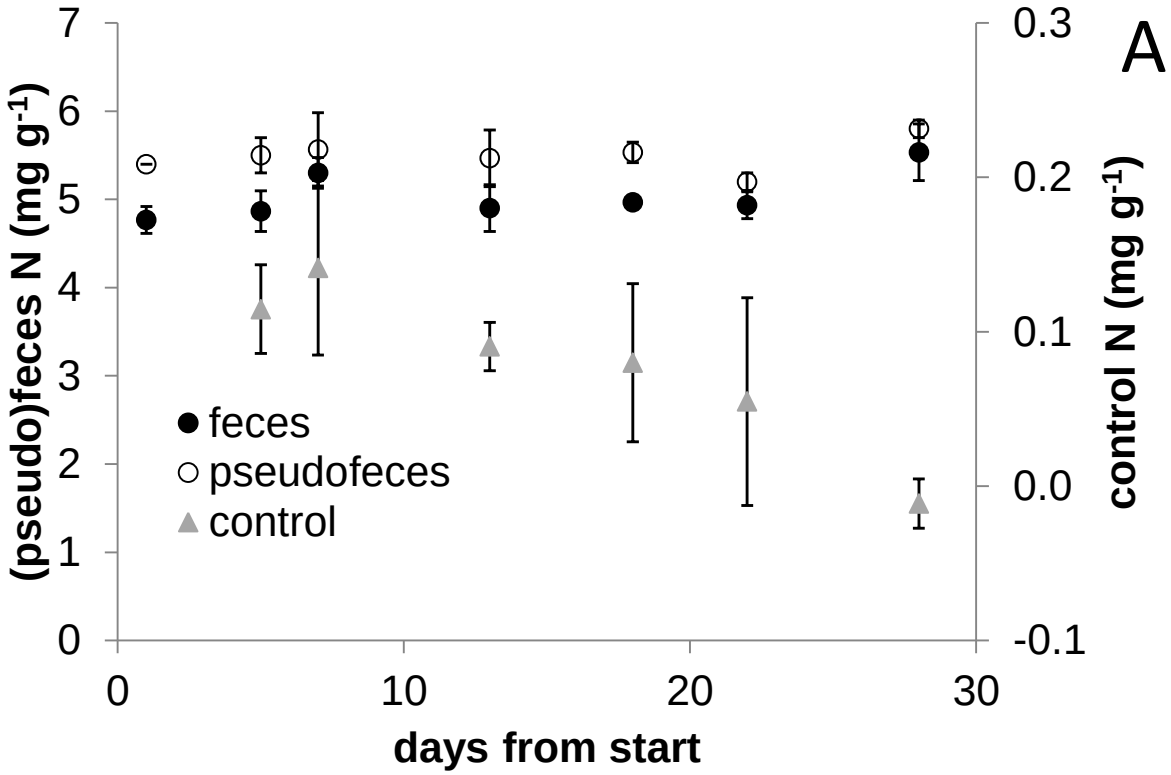


Figure 4. Dissolved organic nutrient quantity throughout the incubation period (element mass standardised to dry mass of start material in chamber; error bars indicate SD; n=3 for each treatment). Panel A: DON; B: DOP; C: DOC. DOC was not measured in control chambers. Straight lines represent significant linear regression trends for pseudofeces over the first 18 days (non-significant trends omitted).

Figure 5


A
(pseudo)feces N (mg g-1)
control N (mg g-1)
days from start
feces
pseudofeces
control
0
1
2
3
4
5
6
7
-0.1
0.0
0.1
0.2
0.3
0
10
20
30

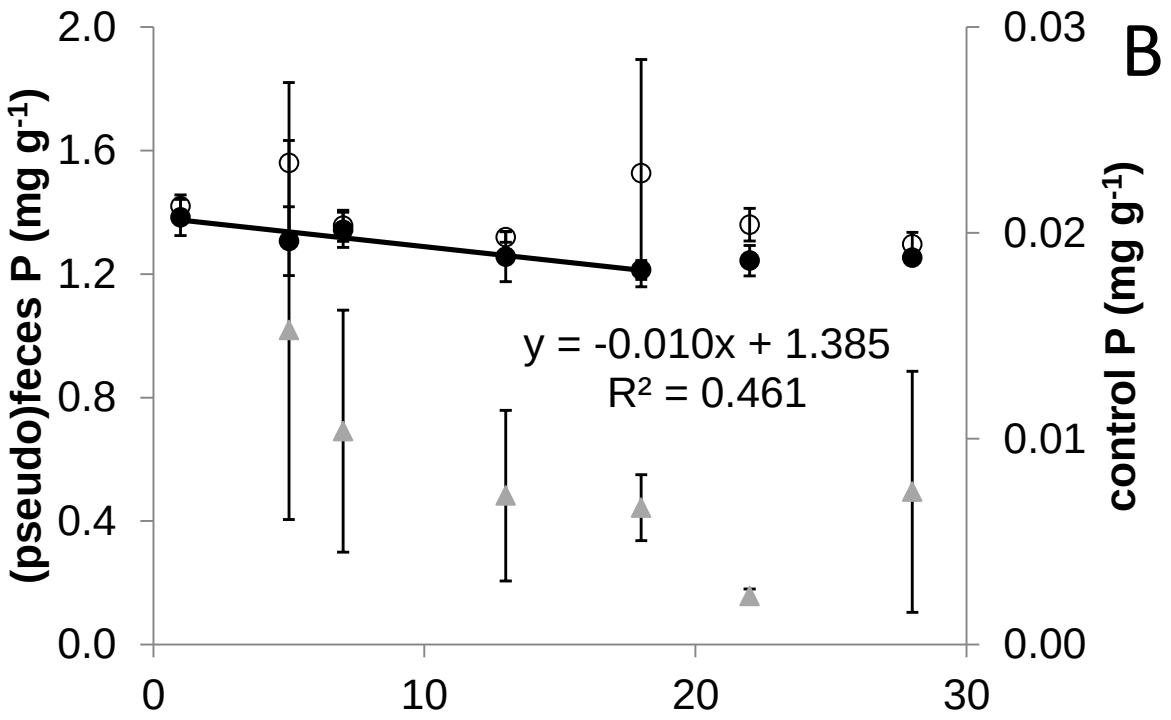


Figure 5. Particulate organic N (panel A) and P (panel B) quantities (element mass standardised to dry mass of start material in chamber; error bars indicate SD; n=3 for all treatments). Straight line represents significant linear regression trend for feces P over the first 18 days (non-significant trends omitted). Control data not available for day 1.

Table 1

Table 1. Dry mass and organic content (day 0; n=5) and nutrient content (day 1; n=3; but for Si n=2) of initial particulate material added per chamber. *: feces and pseudofeces significantly different.

	particulate material added		nutrient content (mg g^{-1} DW)			
biodeposit type	**dry mass (mg)***	**organic content* (%)**	**C***	**N***	**P**	**Si***
feces	485.6 ± 60.4	20.3% ± 2.2%	51.8 ± 0.3	4.8 ± 0.2	1.38 ± 0.06	42.0 ± 1.5
pseudofeces	549.5 ± 43.1	25.9% ± 0.1%	54.9 ± 1.4	5.4 ± 0.0	1.42 ± 0.04	31.2 ± 0.3

Table 2

Table 2. Regression equations describing release of DIN, phosphate and silicate, standardised to 1 g dry mass of start material, after correction for controls and fitted through the origin. Nutrient release rates are calculated by derivative on day 1 and expressed as daily release in per cent of initial amounts.

parameter	feces (mg element g^{-1} DW)	(% d^{-1})	pseudofeces (mg element g^{-1} DW)	(% d^{-1})
DIN	0.035*day	0.73	0.037*day	0.69
phosphate	0.007*day	0.48	0.006*day	0.44
silicate	$0.572*day^{0.886}$	1.21	$0.283*day^{0.930}$	0.84

Table 3

Table 3. Comparison of stoichiometric ratios of nutrients: in feces and pseudofeces chambers on day 1 of incubations; regenerated from biodeposits on day 1; in the Oosterschelde estuary ecosystem at the time of the experiment; and Redfield's ratios.

		N:P	N:Si	Si:P
feces/pseudofeces day 1	feces	7.6	0.22	34.8
	pseudofeces	8.4	0.30	28.4
regenerated nutrients (dissolved inorganic)	feces	11.4	0.14	83.4
	pseudofeces	13.3	0.26	43.4
Oosterschelde average (dissolved inorganic)		3.2	0.70	4.8
Redfield ratio		16	1.07	15