

# Effects of the Invasive Freshwater Mussel *Limnoperna fortunei* on Sediment Properties and Accumulation Rates

Running title: Effects of *Limnoperna fortunei* on sediments

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## Key points:

- The invasive freshwater mussel *Limnoperna fortunei* strongly enhances sediment buildup rates and their contents in organic matter and total nitrogen
- Ecosystem-wide effects of these modifications are likely very significant, especially for soft-bottom, infaunal organisms

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**Abstract** Since its introduction into South America around 1990, the freshwater bivalve *Limnoperna fortunei* (the golden mussel) has spread rapidly, and is now a dominant component of the benthic and periphytic fauna in many rivers, lakes and reservoirs. Sizable impacts of this non-indigenous species on nutrient recycling, plankton abundance and composition, and trophic relationships with fishes have been reported, but its effects on the sediments have received little attention. In this work, we use eighteen 20 L flow-through experimental units with and without mussels where changes in the mass and characteristics of the sediments accumulated throughout a yearly cycle in monthly, biannual and annual intervals are analyzed. Experimental units with mussels yielded almost two times more sediments than units without mussels, and contained significantly higher loads of organic matter (OM) and total N. Total P was not affected by the presence of mussels. Sediments accumulated in the biannual and annual experimental units agreed well with the yields of the monthly units, but the vertical stratification of organic matter, N and P was unpatterned. Seasonal changes in the volume of total sediments, biodeposits, and their OM and N contents, were positively associated with ambient water temperature and with intermediate (~150-250 NTU) turbidity. Our results suggest that ecosystem-wide modifications in the living conditions of the benthic epi- and infaunal organisms in waterbodies invaded by the mussel are likely significant, although variable locally, regionally and across taxa.

**Plain language summary** In the last decades, thousands of plants and animals have spread into new marine, freshwater and terrestrial habitats through human-mediated activities. Worldwide, freshwater ecosystems seem to have suffered the most. The Asian golden mussel, a small (~2-3 cm) bivalve mollusc, was introduced in South America around 1990, and by 2017 it was present, often at densities over 200,000 individuals per square meter, in many rivers, lakes and reservoirs throughout five countries. The mussel, which lives attached to hard substrates, is presently a major biofouling nuisance for many industrial installations, and has been shown to affect pre-existing biological interactions significantly. In this work we show that, in the estuary of the Río de la Plata (South America), the golden mussel doubles the rates of sedimentation of suspended particles, and strongly enhances their contents in organic matter and nitrogen. These effects are likely to increase the rates of accretion along the Río de la Plata estuary front, as well as to modify the makeup of the communities that dwell in the sediments. The golden mussel is expected to continue expanding northwards, and its impacts will likely affect many of the new waterbodies colonized.

*Keywords:* *Limnoperna fortunei*, Golden mussel, impact, sediments, organic matter, Nitrogen, Phosphorus

## 1. Introduction

The presence of filter-feeding species has long been recognized as a key factor that strongly influences the accumulation rates and characteristics of sediments (Dame & Olenin, 2005; Jørgensen, 1990). However, while the effects of indigenous, natural filter-feeders are the result of interactions that evolved throughout thousands or millions of years, those of species cultured (shellfish farms) or introduced by man (non-indigenous species) may disrupt pre-existing conditions over very short time-scales (Nalepa & Schloesser, 2014; Shumway, 2011). In this respect, bivalves are particularly important, both because they are the object of extensive commercial farming worldwide (Shumway, 2011), and because they include a large proportion of the invasive invertebrates (Karatayev et al., 2009).

Several previous surveys investigated the effects of various filter-feeding molluscs on the sediments, including native and introduced species, as well as cultured bivalves. While there is general agreement that total sediments, organic matter and N are enhanced in the presence of the mussels, the behavior of P, the proportions and long-term fate of these biodeposited sediments, and their ultimate effects on bottom properties and buildup rates over large areas are controversial (Crawford et al., 2003; Gergs et al., 2009; Roditi et al., 1997; Ruginis et al., 2014; Shumway, 2011; Zaiko et al., 2010).

During the last ~60 years *Limnoperna fortunei* (the golden mussel), native to southern China, has been introduced in Hong-Kong, Korea, Taiwan, Japan and South America (Boltovskoy & Correa, 2015). In South America, where it was first recorded in the Río de la Plata Estuary around 1990, it spread rapidly upstream the Paraná and Uruguay rivers, and is presently a dominant component of the benthic and epiphytic fauna in two major watersheds (Río de la Plata and São Francisco) and several smaller ones throughout five countries (Argentina, Bolivia, Brazil, Paraguay, and Uruguay) (Barbosa et al., 2016; Oliveira et al., 2015). The introduction of the golden mussel has become an important biofouling nuisance for industrial plants that use freshwater from the colonized waterbodies (Boltovskoy et al., 2015a), and is reshaping biological interactions. Investigation of the impacts of this mussel on the environment and on other organisms has chiefly centered on nutrient recycling, plankton abundance and composition, and trophic relationships with fishes (Boltovskoy, 2015). Effects on the invertebrates associated with its colonies were assessed comparing the fauna present in mussel beds or druses, to nearby areas of the same substrates or sites barren of mussels (Karatayev et al., 2010; Sardiña et al., 2011; Sylvester & Sardiña, 2015), but no previous research focused on the changes that *L. fortunei* beds engender in the sediments.

We address five questions in this survey. (1) How much of the sediments and sestonic organic matter (OM), N and P settling to the bottom is associated with the mussels' presence? (2) Which are the ambient conditions that modulate this process? (3) How much OM, N and P is sequestered in the sediments? (4) How do these materials behave in the sediments (up to) one year after burial? and (5) Which are the potential effects of the mussel-modified sediments on the benthic biota?

## **2. Material and methods**

### **2.1 Regional setting**

This work was conducted on the premises of the water treatment plant AySA (Agua y Saneamientos Argentinos Sociedad Anónima), in Buenos Aires (Argentina), which provided a permanent raw water supply to our experimental device (Experimental Units, or EU henceforth; Figures 1, 2). The plant processes water from the Río de la Plata Estuary, which is gravitationally fed through a 5 m diameter pipe from an intake located ~1 km offshore (34.54°S, 58.42°W). At this location the water is fresh year-round (<3 PSU - Practical Salinity Units) and contains high loads of suspended solids (SS; ~100 mg L<sup>-1</sup>) (Bazan & Arraga, 1993). The estuary, with a catchment area of 3.1 million km<sup>2</sup> and a mean discharge of ~25,000 m<sup>3</sup> s<sup>-1</sup>, is chiefly fed by two major rivers: the Paraná River (~74% of the input) and the Uruguay River (~23%) (Framiñan et al., 1999). Seasonally, discharge values are normally highest in March-June, which usually coincides with peaks in total SS, but SS may also increase after peak discharge periods, when flood water leaves the inundated flood plain (Depetris et al., 2005), as well as during periods of strong winds, which result in vertical turbulence and resuspension of bottom deposits (Moreira et al., 2013).

### **2.2 Experimental setup and sample analyses**

The experimental design consisted of eighteen 20 L EU enclosed in a box covered with a removable lid (Figure 1). Each EU received a constant supply of estuary water at a rate of ~0.8-0.9 m<sup>3</sup> d<sup>-1</sup> (~556-625 mL min<sup>-1</sup>) from a head tank by gravity, which overflowed through a nozzle at the top of each EU (Figure 2). Half of the EU contained a meshwork basket suspended below the water surface (Figure 2a) with 87-255 (mean: 199) adult (>98% between 8 and 28 mm in length) mussels collected ~2-3 days before from nearby locations along the coast of the estuary and rinsed with tap water (EU with mussels, or +M henceforth). Baskets were placed near the water surface (rather than close to the bottom) in order to avoid their burial due to sediment accumulation, especially in the biannual and annual units. The resulting mussel densities were 1318-3864 (mean: 3015) ind. m<sup>-2</sup>, which

are roughly within the range of those observed in nature (see Discussion below). The other half of the EU were barren of mussels (controls; CTR henceforth). In order to minimize disturbing and injuring the animals, clumps of mussels of approximately equal sizes were used (rather than isolated individuals). The size of the mussels varied between periods (Table S1 in the supporting information), for which reason our results were normalized to total mussel tissue dry weight (MTDW). Nevertheless, filtration rates and the rates of production of feces and pseudofeces are not homogeneous across sizes (Sylvester et al., 2005), which may involve some ambiguities in our estimates. On the other hand, because the experimental animals used were collected in the field shortly before each experimental period, their sizes are representative of the dominant population size-structure at that particular time of the year and water temperature, mimicking natural conditions better than if animals more similar in size were used. Upon termination of each monthly experimental period, animals were removed, counted, measured, and their MTDW was estimated using the size to dry weight relationships proposed by Sylvester et al. (2007a):

Mussels <10 mm:  $MTDW (g) = 0.000119 \times e^{0.416 \times \text{mm shell length}}$

Mussels >10 mm:  $MTDW (g) = 0.00396 \times e^{0.113 \times \text{mm shell length}}$

The experiment was deployed on 4 Dec 2014 and terminated 362 days later, on 1 Dec 2015, yielding a total of 66 monthly observations: eleven 25-35 d periods (the 19 d interval between 15 Oct and 3 Nov 2015 was lost due to technical problems), 12 biannual, and 6 annual (Figure S1 in the supporting information).

Of the 18 EU, six (3 +M and 3 CTR) were used for monitoring monthly (25-35 d) changes, six had a biannual (173 and 189 d) duration, and six were maintained throughout the entire experimental period (362 d) (Figure S1 and Table S1 in the supporting information). In the biannual and the annual +M, mussels were replaced every two months to ensure their vitality, minimize the retention of dead organisms, and following the dominant size-structure in nature. Their number, size and biomass were estimated as described above on each replacement event and at termination. Each period (monthly, biannual and annual) and condition (with and without mussels) was repeated simultaneously in triplicate in randomly assigned EU. Mortality in the +M was extremely low, never exceeding ~3% of the animals. These dead mussels were excluded from our estimates, which may have introduced a small bias because they might have been represented by dead individuals retained within the clumps of living ones at the start of each period, or by individuals that died during the experiment.

Water flow into each EU was monitored throughout the experiment. In total, 710 measurements of water flow were performed, both on and between sampling dates. Differences between the 3 +M and the 3 CTR replicates of each period were generally <15%. Differences between successive periods were higher (up to 35%, mean: 13%), for which reason total sediment volumes were adjusted to account for these variations (see below and Table S1 in the supporting information).

Water properties were measured at the intake at the start and end date of each monthly experiment (Figure S1 in the supporting information), as well as at irregular intervals in between: temperature (°C), pH, conductivity ( $\mu\text{S s}^{-1}$ ), dissolved oxygen ( $\text{mg L}^{-1}$ ). Turbidity (Nephelometric Turbidity Units - NTU) was assessed at the intake into the device and at the outlet of each EU. For temperature, in addition to the periodic measurements, we also recorded the values at 30 min intervals by means of miniature temperature, battery-powered data loggers ("thermo buttons"; model 25L, Proges Plus, Willems, France), positioned either at mid-depth (two EU), or in the vicinity of the surface and next to the bottom (one EU) (Figure 2).

At the end of each monthly period, all sediments accumulated in the monthly EU were recovered and subsampled for assessment of their dry weight (60°C until constant weight), organic matter (OM) contents (in %, loss on ignition: difference between dry weight and weight after igniting for 3 h at 500°C). Total P (ascorbic acid method) and total N (cadmium reduction method) in the sediments were assessed spectrophotometrically after digestion with potassium persulfate (Valderrama, 1981). For the biannual and annual EU, total dry weight was estimated as above, but %OM, and total P and N were assessed at 2 cm intervals using cores 2.5 cm in diameter obtained from the EU (3-9 cores from each EU; Figure 2). Except where noted otherwise, for the biannual and annual EU, %OM, P and N are based on the mean values of all 2 cm intervals in the corresponding cores.

Because experimental periods, mussel biomass, and the volumes of water supplied to the EU were not identical, total sediments accumulated in the +M were normalized to 5.2 g of MTDW (the average for all experiments), one day, and 1 m<sup>3</sup> of water per day, and their properties (%OM, P, N) to 5.2 g of MTDW. In the CTR, the mass of sediments was normalized to one day and 1 m<sup>3</sup> of water per day.

This experimental design allowed differentiating passively settling sediments (in the CTR; "passive sediments" henceforth), from passive + biologically-mediated sedimentation ("passive+biodeposited sediments" henceforth), and, by subtraction, sediments accumulated



due to the production of feces and pseudofeces by the mussels ("biodeposited sediments" henceforth). Differences in sedimentation rates between CTR and +M are unlikely to have been affected by hydrodynamic effects due to the presence of the cages (EUM) because the cages occupied only ~1% of the total volume of the EU.

SS in the water ( $\sim 100 \text{ mg L}^{-1}$ , with peaks  $>600 \text{ mg L}^{-1}$ ; Simionato et al., 2011) were not assessed in our work, but in the Río de la Plata total SS are tightly coupled with NTU (total  $\text{mg L}^{-1} \text{ SS} = 0.73 \text{ NTU}$ ,  $R = 0.93$ ; Moreira et al., 2013); we therefore used this conversion factor when comparing our results with literature data where total SS were informed.

Analyses of relationships between environmental variables and experimental results were based on bivariate methods (correlations, t-tests). Multivariate treatments were hindered by the strong collinearity between several of the independent variables considered (e.g.,  $\text{O}_2$ , water temperature, and mean mussel size; conductivity and turbidity). ANOVA or Kruskal Wallis-Mann Whitney were used to compare mean differences in the response variables (total and biodeposited sediments, %OM, P, N) for the monthly periods with different temperature and turbidity offsets. Differences in turbidity at the outlet of the EU as compared with those at the intake were assessed with ANOVA and ANCOVA. Parametric methods were used when raw or transformed data met the assumptions required. When test assumption were not met, the statistical indicators were based on ranked values (non-parametric) (program PAST, Hammer et al., 2001).

### **3. Results**

#### **3.1 Environmental conditions**

Water temperature followed the usual seasonal trend for the upper Río de la Plata Estuary, with highest values ( $\sim 25\text{-}28^\circ\text{C}$ ) in December-March, and lowest ( $\sim 12\text{-}16^\circ\text{C}$ ) in June-September (Figure S2 a in the supporting information). In the EU, temperature varied little with depth. The EU provided with two temperature data loggers (one near the surface and one next to the bottom, Figure 2) yielded a mean daily difference of  $0.32^\circ\text{C}$  ( $\text{SD} = 0.33$ ). Day-night differences in temperature were also relatively small (mean for the entire experimental period  $\sim 0.7^\circ\text{C}$ ), with highest values ( $\sim 1\text{-}2^\circ\text{C}$ ) in the warmest months, and lowest ( $\sim 0.6^\circ\text{C}$ ) during the colder period (Figure S3 in the supporting information).

Dissolved oxygen was always high ( $>6 \text{ mg L}^{-1}$ ) and close to saturation (75 to  $>100\%$ , mean: 90%). Variations in pH were small (generally 6.5 to 7.5; Figure S2 b in the supporting



information). Conductivity and turbidity depicted similar trends, with highest values in the fall-winter (April-June; Figure S2 c in the supporting information).

Temperature was highly correlated with O<sub>2</sub> (Spearman's R=-0.895, P<0.001), which simply reflects the fact that oxygen is more soluble in colder waters; and with mussel length (Spearman's R=-0.691, P=0.019). *L. fortunei* reproduces during the warmer months and therefore high water temperatures are associated with higher proportions of juveniles and lower mean mussel size. Conductivity generally paralleled turbidity (Figure S2 c in the supporting information), but the correlation was not significant (Spearman's R=0.509, P=0.110). We therefore centered our attention on the two independent variables that were not associated throughout the experimental period (water temperature and turbidity; Spearman's R=0.067, P=0.100, N=613), and that have been shown in previous surveys to affect mussel filtration rates most clearly (Boltovskoy et al., 2015b; Tokumon et al., 2015).

### 3.2 Sediment accumulation rates

Sediment accumulation varied greatly throughout the year (Figure 3a, 4a, b). Mussels significantly increased sedimentation (Mann-Whitney, P<0.001), where mean +M rate was 40.5 g DW d<sup>-1</sup>, relative to the CTR mean of 21.6 g DW d<sup>-1</sup> (Figure 3a, Table S1 in the supporting information). On average for all the experimental periods, biodeposited sediments (i.e., +M minus CTR) represented 47% (range: 7-65%) of the totals (i.e., passive+biodeposited sediments).

Turbidity at the inlet and outlet of all EU were correlated, but the slopes of the +M and CTR were significantly different (ANCOVA, p<0.011). CTR aligned closer to the 1:1 ratio than +M (Figure S4 a in the supporting information). In the CTR, the outlet was 74% of the inlet value, while the +M outlet was significantly lower at 62% of the inlet (Mann-Whitney P<0.001; Figure S4 b in the supporting information).

Although monthly values for the +M paralleled those in the CTR (Figure 5a), in the former they were significantly associated with temperature, whereas in the CTR they were not (Figure 4a). Ambient turbidity did not affect sediment accumulation rates significantly..

### 3.3 Biodeposited sediments

In the +M, biodeposited sediments were clearly (positively) associated with temperature (Figure 4c). Association with turbidity was also significant, but highest values coincided with

periods when ambient turbidity was ~150-250 NTU, decreasing below and above these values (Figure 4d).

### 3.4 Organic matter (OM)

Proportions of OM were almost invariably higher in the +M than in the CTR (Figure 3b). On average for all the EU, %OM in the CTR was 69% of that in the +M. Differences between +M and CTR were statistically significant (mean for +M: 4.8%, CTR: 3.3%,  $P=0.011$ , t-test). The monthly means were generally similar with those recorded in the annual EU (Figure 3b).

Although mean differences at temperatures above and below 20°C differed more in the +M than in CTR, neither set of monthly units (+M and CTR) showed significant associations between %OM and water temperature (Figure 4e).

In the CTR, ambient turbidity variations had no effect on %OM, whereas in the +M %OM tended to increase with increasing turbidity at the intake up to values ~150-250 NTU, dropping thereafter (Figure 4f). In the biannual and annual EU, the vertical distribution of %OM down to 6-8 cm (CTR), and to 12-18 cm (+M) was homogeneous and showed no trend (Figure 6a-c).

### 3.5 Total P

Total P differed little between +M and CTR (Figure 7a) (mean for +M=0.215, mean for CTR=0.206 mg g<sup>-1</sup> DW; Man-Whitney test  $P=0.482$ ), although month-to-month variability was significantly higher in the +M (SD=0.051) than in the CTR (SD=0.026) (F test  $P=0.044$ ). In the +M, total P was positively associated with temperature, but not in the CTR, where the correlation was non-significant (Figure 4g).

Associations with turbidity were marginally significant (negative) in the CTR, and non-significant in the +M (Figure 4h). Monthly variations in +M and CTR were not associated (Figure 5c). In agreement with the monthly results, in the biannual and annual EU the vertical distribution of total P varied randomly between +M and CTR, as well as with depth (Figure 6d-f).

### 3.6 Total N

N concentrations were higher in the +M than in the CTR in all the monthly EU (Figure 7b), as well as at all depths in the cores retrieved from the biannual and annual EU (Figure 6g-i).

Monthly means differed significantly ( $P=0.002$ , Mann-Whitney test), but the temporal trends in the +M and the CTR clearly paralleled each other (Figure 5d). In the +M (but not in the CTR), total N was positively correlated with temperature (Figure 4i). Cores from the biannual and annual EU showed no vertical pattern (Figure 6g-i).

## 4. Discussion

### 4.1 Sediment accumulation rates

Our results support the notion that *L. fortunei* has a major effect on sedimentation rates and the properties of the resulting deposits (Figures 3, 7), and that strongest enhancements of biodeposited sediments occur at times of higher water temperatures (Figure 4). The presence of mussels almost doubled sediment accumulation rates, and significantly increased their contents of OM and total N.

Because of differences between our experimental settings and natural conditions, the sediment accumulation rates in our EU are difficult to compare with field data. However, a rough estimate suggests that our values are a reasonable proxy of actual sedimentation rates in the estuary. Mean sediment accumulation rates in the +M were  $3.5 \text{ cm m}^{-2} \text{ y}^{-1}$ , whereas in the CTR they averaged  $1.9 \text{ cm m}^{-2} \text{ y}^{-1}$ . These values are comparable with those estimated using cores from the upper Río de la Plata Estuary dated with  $^{210}\text{PB}$  and  $^{137}\text{Cs}$ : around  $0.25\text{-}2.6 \text{ cm y}^{-1}$  (Bonachea et al., 2010; Di Gregorio et al., 2007; Schuerch et al., 2016), and up to over  $5 \text{ cm y}^{-1}$  in some near-shore areas (Colombo et al., 2007). A rough approximation comparing historical (since 1778) and present-day elevation data for the mouth of the Paraná River Delta (Sarubbi, 2007) suggests vertical growth rates around  $1\text{-}3 \text{ cm y}^{-1}$ .

The amount of sediments accumulated in the biannual and annual EU was somewhat lower than the sum of the corresponding monthly EU. Aside from the circumstance that the monthly EU missed a 19 days interval (Figure S1 in the supporting information), this difference is probably due to the fact that in the biannual and annual EU the top of the sediment layer was closer to the outlet, and therefore a larger proportion of the incoming particles were flushed out. However, this effect does not seem to have affected comparisons between the monthly EU, where the top sediment layer was much farther away from the outlet ( $\sim 25\text{-}27 \text{ cm}$ ) than in the biannual and annual ones ( $\sim 5\text{-}10 \text{ cm}$ ), and the ratio of turbidity at the intake vs. turbidity at the outlet was uncorrelated with sediment buildup rates (+M, Spearman's  $R=-0.367$ ,  $P=0.336$ ; CTR, Spearman's  $R=-0.409$ ,  $P=0.212$ ).

Comparison of our results with previous data on contrasts between passive and passive+biodeposited sedimentation rates show roughly comparable values. On the basis of four 24 h experiments conducted in chambers with and without zebra mussels (*Dreissena polymorpha*) in the Hudson River, Roditi et al. (1997) estimated a mean rate of biodeposited sediments of  $\sim 55 \text{ mg DW mussel}^{-1} \text{ d}^{-1}$ , whereas our values were  $\sim 97 \text{ mg DW mussel}^{-1} \text{ d}^{-1}$ . The mean size of the mussels used in the two studies was very similar ( $\sim 17 \text{ mm}$ ), but the amount of SS is almost ten times lower in the Hudson River ( $\sim 10 \text{ mg DW L}^{-1}$ ), than in the Río de la Plata Estuary ( $\sim 100 \text{ mg DW L}^{-1}$ ). Suspended solids and species-specific biofiltration differences likely account for our higher values. Previous studies with zebra mussels support this assumption: at  $50\text{-}87 \text{ mg DW SS L}^{-1}$  biodeposition rates of  $100\text{-}164 \text{ mg DW mussel}^{-1} \text{ d}^{-1}$  were reported (Klerks et al., 1996; Reeders & bij de Vaate, 1992). Mussel pumping rates and the production of feces and pseudofeces vary in response to the concentrations of SS in the water, with both positive (Gergs et al., 2009; Maclsaac & Rocha, 1995; Nalepa et al., 1991) and negative (Sprung & Rose, 1988) relationships having been reported, but the range of inorganic SS loads is probably a major factor to which the mussels respond bimodally. For *L. fortunei*, Tokumon et al. (2015) found that filtration rates increase between 0 and  $100 \text{ mg DW SS L}^{-1}$ , dropping sharply at values above  $500 \text{ mg DW SS L}^{-1}$ . In the Salto Grande Reservoir (Argentina-Uruguay), at values around  $5\text{-}15 \text{ mg DW SS L}^{-1}$ , Cataldo et al. (2012b) found that, after 35 d, 400 L mesocosms with *L. fortunei* accumulated 5-6 times more sediments than mesocosms without mussels, which is higher than our values with Río de la Plata water ( $\sim 2$ ), where SS were up to  $>270 \text{ mg L}^{-1}$  (Table S1 in the supporting information). In our +M, biodeposited sediments showed an increasing trend up to ambient turbidity values  $\sim 150\text{-}250 \text{ NTU}$  ( $\sim 110\text{-}180 \text{ mg DW SS L}^{-1}$ , Moreira et al., 2016), decreasing at higher values (Figure 4d), which is in line with the results of Tokumon et al. (2015).

#### 4.2 Organic matter and total N

With one exception, %OM and N were always significantly higher in the presence of mussels (+M) than in the controls (CTR) (Figures 3, 7b). It should be noted that both %OM and N in all the EU were influenced by ambient values as well; although values in the +M were consistently higher than those in the CTR, monthly changes in the EU with and without mussels paralleled each other (Figure 5b, d). Values for %OM in the CTR were closely comparable with information for the surface sediments of the Río de la Plata Estuary (Moreira et al., 2016; Ronco et al., 2008). Our results agree with previous data where biodeposited sediments were found to be highly enriched with OM and/or N when compared with passively deposited sediments (e.g., Graf & Rosenberg, 1997; Hatcher et al., 1994;

Jordan, 1987; Kaspar et al., 1985; Roditi et al., 1997). Bivalve biodeposits can enrich sediment OM and impact N cycling in sediments through increasing direct denitrification (i.e., denitrification of water column nitrate), reducing coupled nitrification-denitrification, or increasing ammonium efflux, depending on overarching factors such as eutrophication and habitat heterogeneity (Smyth et al., 2015).

### **4.3 Total P**

As opposed to OM and N, the concentrations of total P were not higher in the +M than in the CTR (Figure 7a). While the effects of biodeposits on the enrichment of sediments with OM and N were observed in many surveys (see above), in particular those aimed at assessing the environmental effects of shellfish cultures in marine coastal areas (Shumway, 2011), results with P are more controversial (Biswas et al., 2009; Nalepa et al., 1991; Strayer, 2014; Wetzel, 2001). Several studies concluded that biodeposits contain significantly higher loads of P than seston and/or passive sediments (Jordan, 1987; Kleeberg, 2002; Mosley & Bootsma, 2015; Nalepa et al., 1991; Sornin et al., 1986), and that P concentrations in the water column decrease after colonization of the waterbody with filter-feeding mussels (Mayer et al., 2014; Mida et al., 2010). In contrast, many others found no differences in P concentrations in sediments influenced by biodeposited matter with those farther away from biodeposit-producing organisms (Carlsson et al., 2009; Hatcher et al., 1994; Mellina et al., 1995; Zaiko et al., 2010), recorded strong increases in phosphates in the water column in the presence of mussels (Boltovskoy et al., 2009; Cataldo et al., 2012a; Cataldo et al., 2012b), and no association between OM and P in sediments (Tye et al., 2016). These dissimilar results may be related with the lower sediment retention of P than of N (Dillon et al., 1990). Seasonal changes in P loads in the sediments have been recorded in some studies (Mosley & Bootsma, 2015), but not in others (Mazouni et al., 1996; Nalepa et al., 1991). In our data no seasonal trends were found, which may suggest that proportions of P in the water are seasonally more stable than those of OM and N, and/or that biodeposited P is largely released to the water column.

Several mechanisms have been suggested to account for this different behavior of P (as compared with OM and N), including the release of unbound P due to OM-induced oxygen depletion (Newell, 2004; Newell et al., 2005; Shumway, 2011), bioturbation by infaunal organisms, in particular chironomids and mayflies, that can reduce P retention in the sediments and favor its release as bioavailable P into the water column (Biswas et al., 2009; Chaffin & Kane, 2010), differences in the functions of P and N in animal physiology (Vanderploeg et al., 2017), and in the physical and chemical traits of the water and the

sediments (Li et al., 2013; Reynolds & Davies, 2001; Tye et al., 2016). It has been suggested that sediment retention of P bound to the dead shells (rather than to the soft tissue and the biodeposits) of the mussels can take up to 100 y to reach a balance before sediments become a net source of P, whereas until that point sediments are a net sink (Ozersky et al., 2015). However, in the Ca-poor South American waterbodies (Karatayev et al., 2007) empty *L. fortunei* shells have never been observed to accumulate in the sediments, and their dissolution can be even faster than the disintegration of the soft tissue (Boltovskoy et al., 2009).

#### **4.4 Vertical distribution of properties in the sediments**

In the cores obtained from the biannual and annual EU, contrasts between +M and CTR were similar to those observed in the monthly EU, but neither of the variables measured showed consistent vertical patterns (Figure 6). To compare our CTR data for OM with field conditions, we took several 20-30 cm cores in the vicinity of the Río de la Plata Estuary (Pajarito River, Paraná delta, ~5 km upstream from the estuary), and assessed their OM contents in 1 cm intervals. Highest %OM figures (>4%) were recorded at 1 and 12 cm, but down to 26 cm no trend was observed (mean=3.5%, range=2.7-4.3%, Spearman's R of depth in the core vs. %OM: -0.316, P=0.116). In sediments of the upper Río de la Plata Estuary, organic C in the sediments has been reported to drop from ~3% at the surface (5% OM, according to the conversion factor proposed by Hoogsteen et al., 2015) to ~2% (3.3% OM) at 4 cm, oscillating around 2% down to 30-50 cm (Schuerch et al., 2016), which agrees well with our data (mean for +M: 4.8%, CTR: 3.3%; Figures 3, 6, and Table S1 in the supporting information). Previous literature information from freshwater bodies elsewhere shows widely dissimilar results (e.g., Bloesch, 2004; Grenz et al., 1990; Sobczyński & Joniak, 2009; Torres et al., 2012), suggesting that local spatial and temporal conditions result in dissimilar vertical profiles for these attributes.

It should be borne in mind that our survey covered 1 year only, whereas remineralization of the OM can take longer than that, and therefore the proportions of OM ultimately buried as refractory carbon, and especially N, can be lower (Bloesch, 2004). Nevertheless, the fact that the OM contents of our EU compare favorably with downcore field data suggests that our experimental results are not unrealistic.

#### **5 Considerations for scaling up results to predict local and regional impacts**

The effects of *L. fortunei* on the sediments are similar to those reported for the effects of the zebra mussel and other filter-feeding molluscs (Klerks et al., 1996; McCall et al., 1986; Mellina et al., 1995; Roditi et al., 1997; Ruginis et al., 2014; Zaiko et al., 2010), but



extrapolation of our experimental conditions to the field is not unequivocal. Among the most important caveats are: (1) The lack of adequate data on densities of *L. fortunei* over large areas; (2) Uncertainties associated with the ease of resuspension of the passive+biodeposited sediments as compared with that of passive sediments; and (3) Differences in the winnowing and dispersion of the biodeposits in different lotic and lentic waterbodies colonized by the mussel.

Data on *L. fortunei* densities over large areas are extremely scarce. Boltovskoy et al. (2009) assessed *L. fortunei* densities in the reservoir Embalse de Río Tercero (Argentina), reporting figures of up to over 6200 ind. m<sup>-2</sup> (mean for the entire reservoir: 959 ind. m<sup>-2</sup>). The extensive floodplains of many lowland rivers colonized by the mussel in South America (Oliveira et al., 2015) are densely populated by the water hyacinth *Eichhornia crassipes*, whose roots host up to >2760 ind. m<sup>-2</sup> (Musin et al., 2015) (in our EU, mussel densities were ~3000 ind. m<sup>-2</sup>). The rate of production of biodeposits depends on both mussel densities and on the volume of water available to the mussels for filtration. In Embalse de Río Tercero, the average volume of reservoir water per mussel per day is theoretically around 0.4 L (Boltovskoy et al., 2009). In our experiment water was supplied to the EUM at ~6 L mussel<sup>-1</sup> d<sup>-1</sup>, which is higher than the above value, and most probably largely in excess of the volumes that *L. fortunei* can process (<2 L ind.<sup>-1</sup> d<sup>-1</sup>; Boltovskoy et al., 2015b). We therefore anticipate that, in this respect, the experimental conditions used are generally within the range of actual conditions in many South American waterbodies.

Differences in the resuspension rates, winnowing and lateral advection between biodeposited and passively deposited sediments are controversial. Strayer (2014) suggested the mussel biodeposits are heavier than passive sediments and therefore may have a higher tendency to be retained locally, yet other authors found the opposite (McCall et al., 1986; Roditi et al., 1997).

Our results indicate that passive+biodeposited sediments are enriched with smaller-sized particles as compared with passive sediments. The decay in turbidity of untreated, well mixed (all depths) resuspended sediments recovered from the annual EU shows that after 2 h CTR (passive sediments) are ~70% less turbid than passive+biodeposited sediments (+M), indicating that they contain higher proportions of larger, rapidly settling particles (Figure S5 in the supporting information). Indeed, in the passive+biodeposited sediments (+M) the proportions of clay (<4 μm) are two times higher than in the passive sediments (CTR), whereas those of silt (4-60 μm) are ~30% lower (Figure S6 in the supporting information). These differences between the +M and CTR are very highly significant for both



clay and silt ( $P < 0.001$ , Mann-Whitney tests). These contrasts are most probably due to the higher retention of clay in the mussel's mucus-bound feces and pseudofeces, and therefore are conceivably less prone to resuspension. The fate of mussel biodeposits is likely variable. Resuspension may relocate these OM-rich materials and the inorganic particles bound in the feces and pseudofeces, which subsequently settle in lower energy areas; it may disperse them facilitating downstream transport and exportation; or simply return them to the water column where they are ultimately degraded. The balance between these processes is obviously waterbody and site-specific (Boyd & Heasman, 1998; Crawford et al., 2003; Hartstein & Rowden, 2004; Strayer, 2014). In rivers, downstream dispersion probably prevails over local accumulation, whereas in lakes and reservoirs, where currents are weaker, sediments are usually transported from the shallow coastal belt to deeper areas. In the reservoir Embalse de Río Tercero, >98% of the mussel beds are located along the coasts at depths <10 m, which is where hard substrates are available (Boltovskoy et al., 2009). Yet, eight years after *L. fortunei* invaded this reservoir, the coastal rocky fringe was still practically free of sediments, except for a thin layer of loose, highly mobile particles on top of the mussels (Boltovskoy et al., 2009). Thus, rather than accumulating above the mussel beds, the biodeposits produced by these colonies are removed from the coastal belt (where turbulence due to wave action is highest) and settle elsewhere, at depths >10 m, which are almost completely dominated by silt (Boltovskoy et al., 2009). In the Río de la Plata Estuary resuspension is very high, especially in the upper freshwater section where water depths are lowest. However, this section, as well as much of its coasts farther downstream, are densely populated by rooted macrophytes (*Scirpus* spp.), whose growth is probably facilitated by the enhanced nutrient levels in the sediments (Bertness, 1984), and which have been shown to decrease sediment resuspension rates significantly (Horppila & Nurminen, 2005).

Most surveys on the effects of *L. fortunei* on the benthic fauna show that invertebrates that dwell among the mussels are more diverse and abundant than those outside of the mussel colonies (Burlakova et al., 2012; Karatayev et al., 2010; Sylvester et al., 2007b). These differences have been attributed to the enhanced structural complexity represented by the mussel beds and to the mussels' biological activity (Sardiña et al., 2008). On the other hand, studies of bare sediments influenced vs. uninfluenced by the presence of mussels are very scarce and their results are controversial. In a study of soft-bottom communities at different distances from large *L. fortunei* beds, Sardiña et al. (2011) concluded that, at a pristine site, species richness and abundance increased away from the mussels, but at a polluted one the trend was irregular. In several Brazilian reservoirs, areas populated by the non-indigenous bivalves *L. fortunei* and *Corbicula fluminea* were reported to host lower overall diversity and

abundance than areas barren of them, yet the biomass of the non-indigenous bivalves was uncorrelated with that of the native fauna (Pearson's  $R=0.044$ ) (Linares et al., 2017).

The effect of sediment changes on the biota is unresolved, but is likely to be significant and highly variable among locations and taxa. Higher OM in biodeposits, as well as their higher proportions of small-sized particles, may reduce  $O_2$  levels in sediment pore-water (Yamada et al., 2012). However, strong and persistent near-bottom oxygen depletion events are unlikely in the Río de la Plata Estuary because wind-driven vertical mixing of its shallow (mostly  $<10$  m) water column and massive sediment resuspension events are very common (every 12.5 h to 8.5 d; Moreira et al., 2013). Major changes in benthic communities are associated with the percent of time under hypoxic conditions, which is most probably low in the estuary, rather than with instantaneous, short-lived low oxygen events (Cicchetti et al., 2006). Thus, we anticipate that, in the estuary, the facilitating effects of the OM-rich sediments on deposit-feeding organisms outweigh the inhibiting ones of lower dissolved  $O_2$  concentrations. Facilitating effects are probably more marked for motile animals, like fishes, than can move away from the occasionally oxygen-depleted layer. Epi- and infaunal invertebrates permanently restricted to the sediments and highly sensitive to short periods of hypoxia can conceivably suffer a negative impact.

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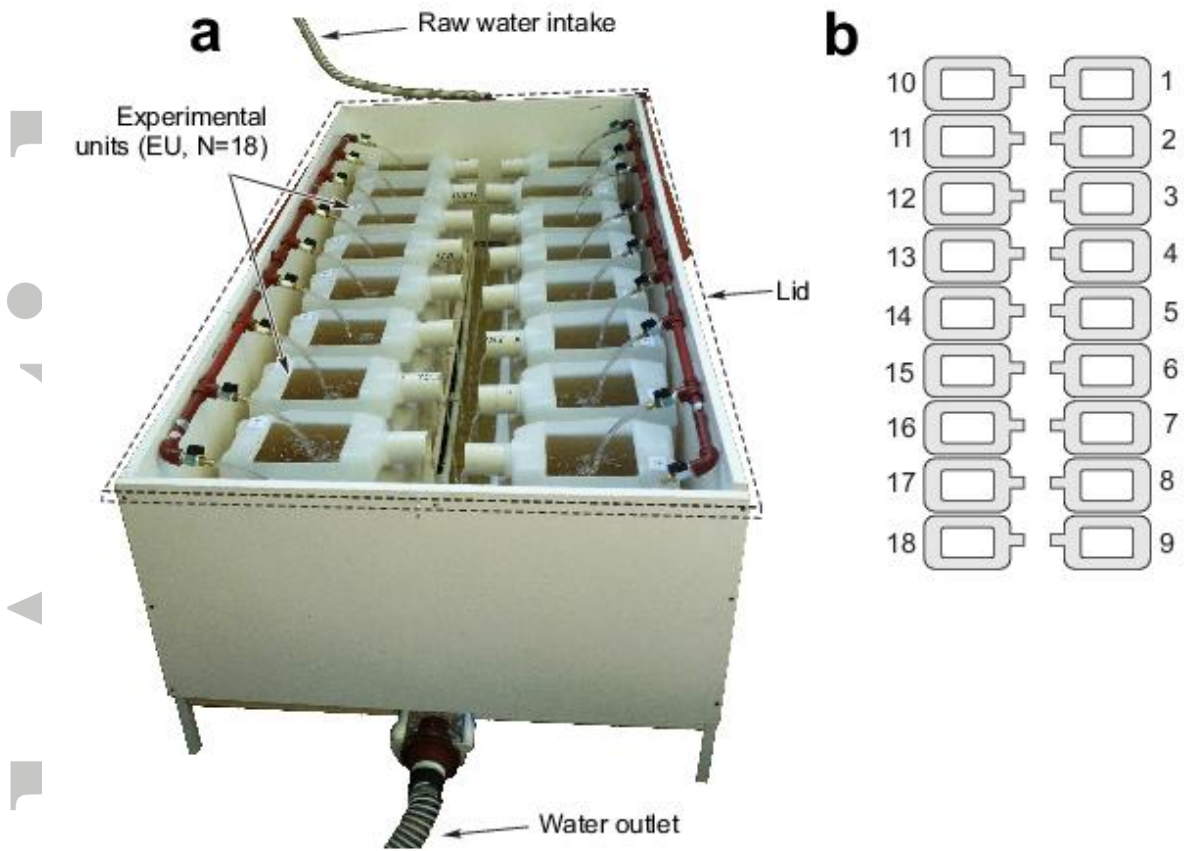
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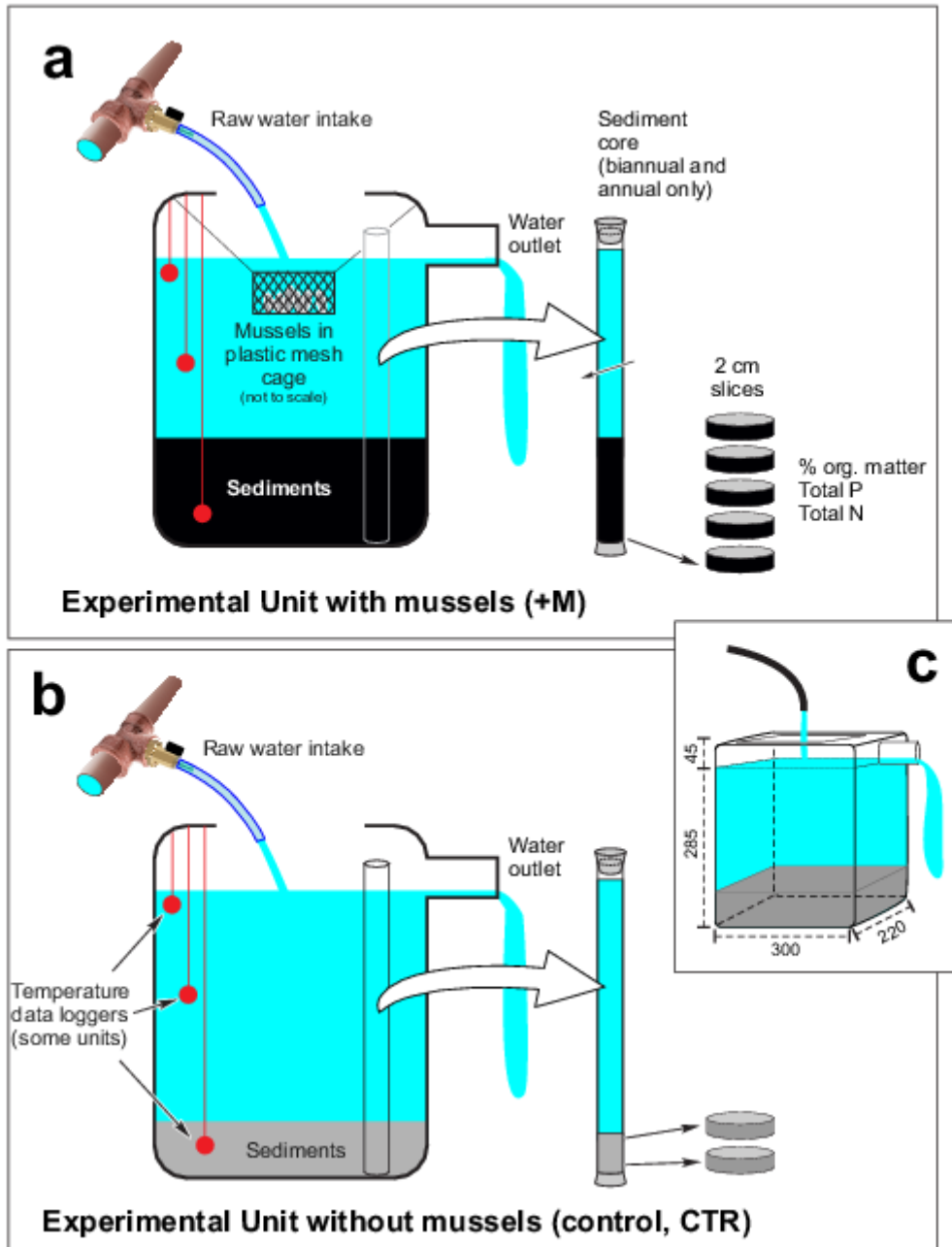
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**Figure 1.** General scheme of the experimental setup (a), and code numbers for the experimental units (b).

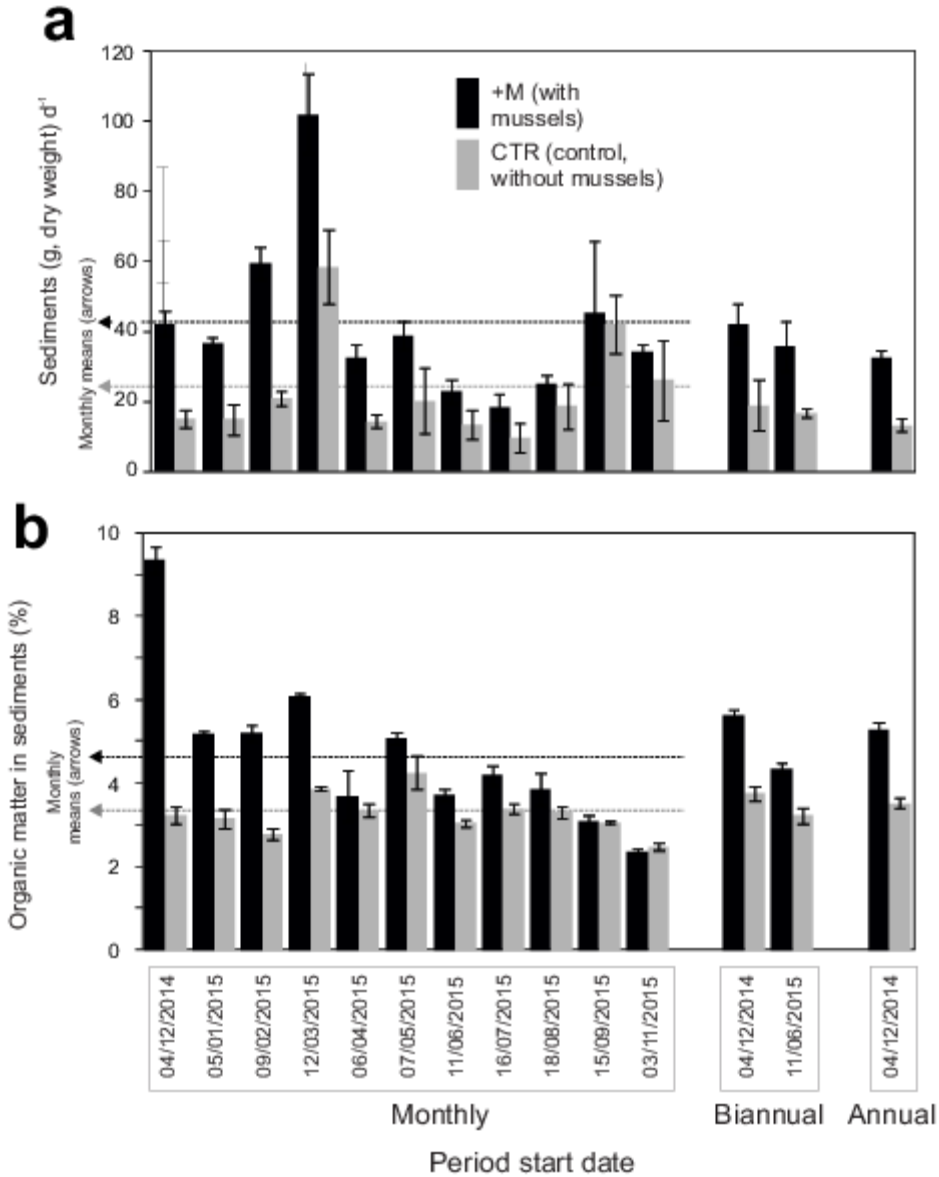
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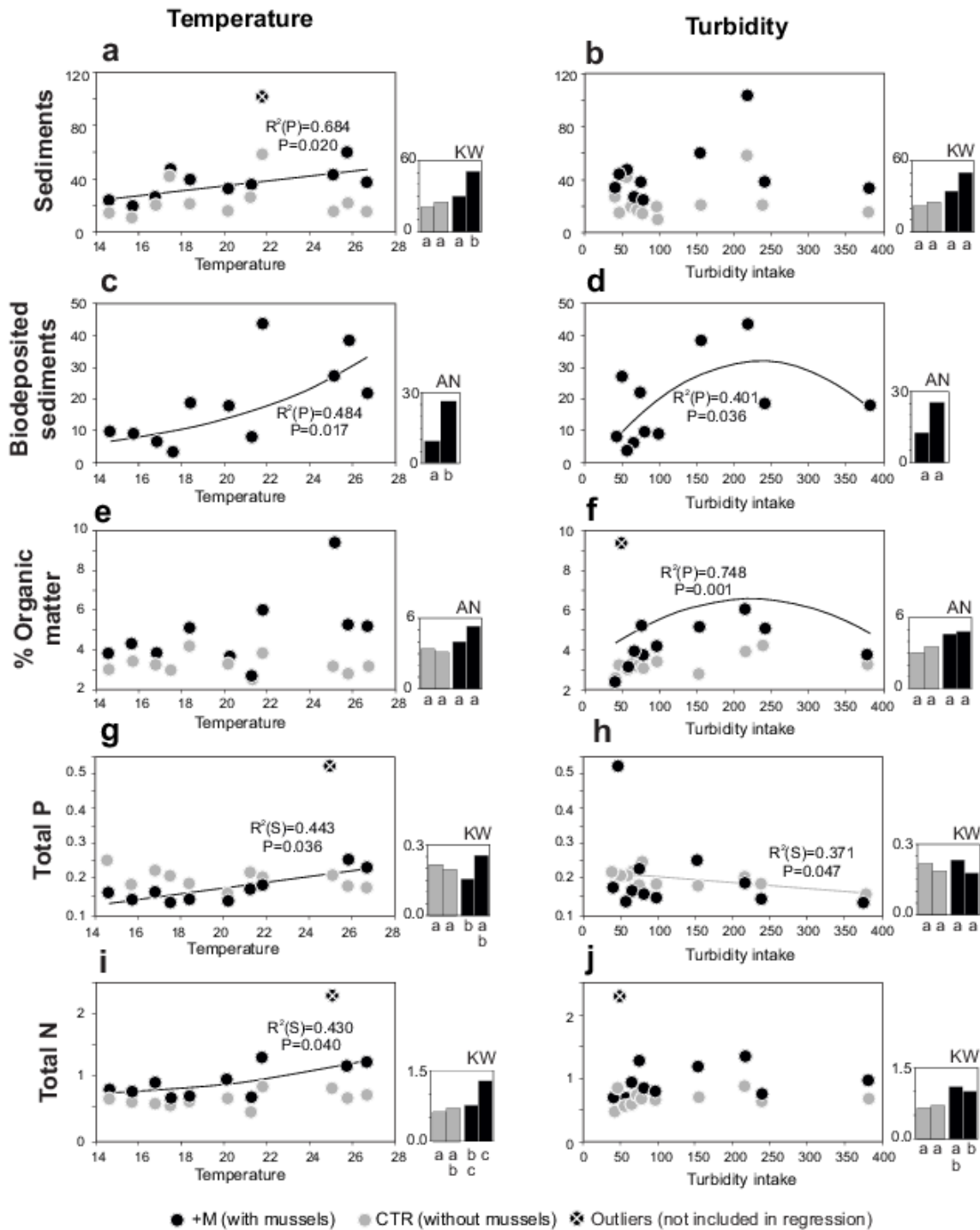


**Figure 2.** Schematic detail of experimental units with mussels (+M, a), and without mussels (controls, CTR, b). c: Perspective view and measurements (in mm) of the experimental units.





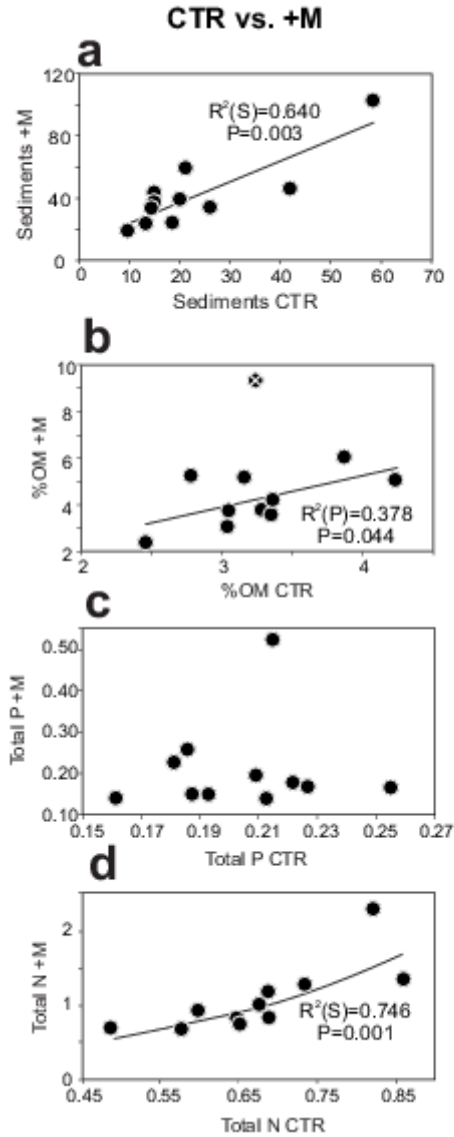
**Figure 3.** Mean and SD of accumulated sediments (a) and % of organic matter (OM) in the sediments (b) in the experimental units. For OM, bimonthly and annual data are means of all 2 cm intervals in the cores obtained from these EU (Figure 6). See Table S1 in the supporting information for data and units.



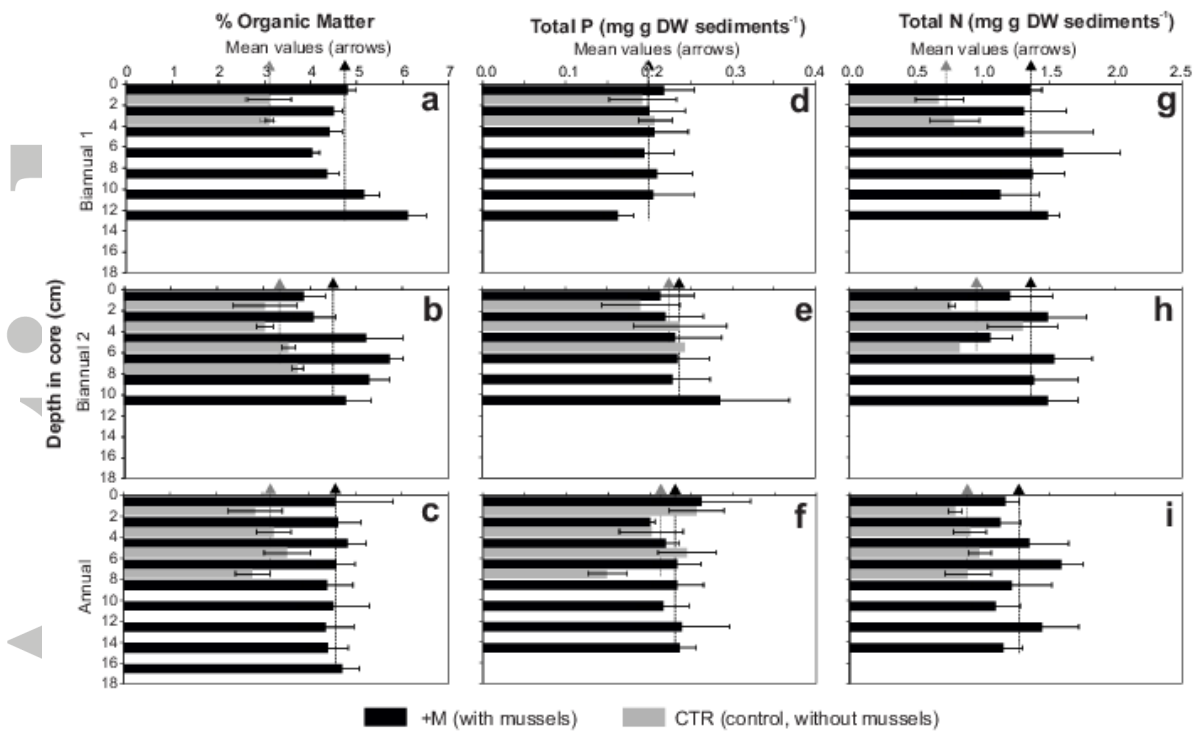
**Figure 4.** Relationships between the variables assessed. Regression lines and R and P values are indicated for the significant ( $P < 0.05$ ) correlations. All datapoints and regression lines are based on actual values, correlation R and P are Pearson's (P) or Spearman's (S; when either variable did not comply with normality). a, g, h: linear; d, f, i: second order polynomial; c: power. Bar graphs show average values for the monthly periods with temperatures below 20°C (N=5) and above 20°C (N=6), or turbidity below 90 NTU (N=6) and above 90 NTU (N=5). Different letters denote significantly ( $P < 0.05$ ) different means (AN: one

way ANOVA and Tukey's contrasts; KW: Kruskal-Wallis test and Mann-Whitney contrasts).  
See Table S1 in the supporting information for data and units.

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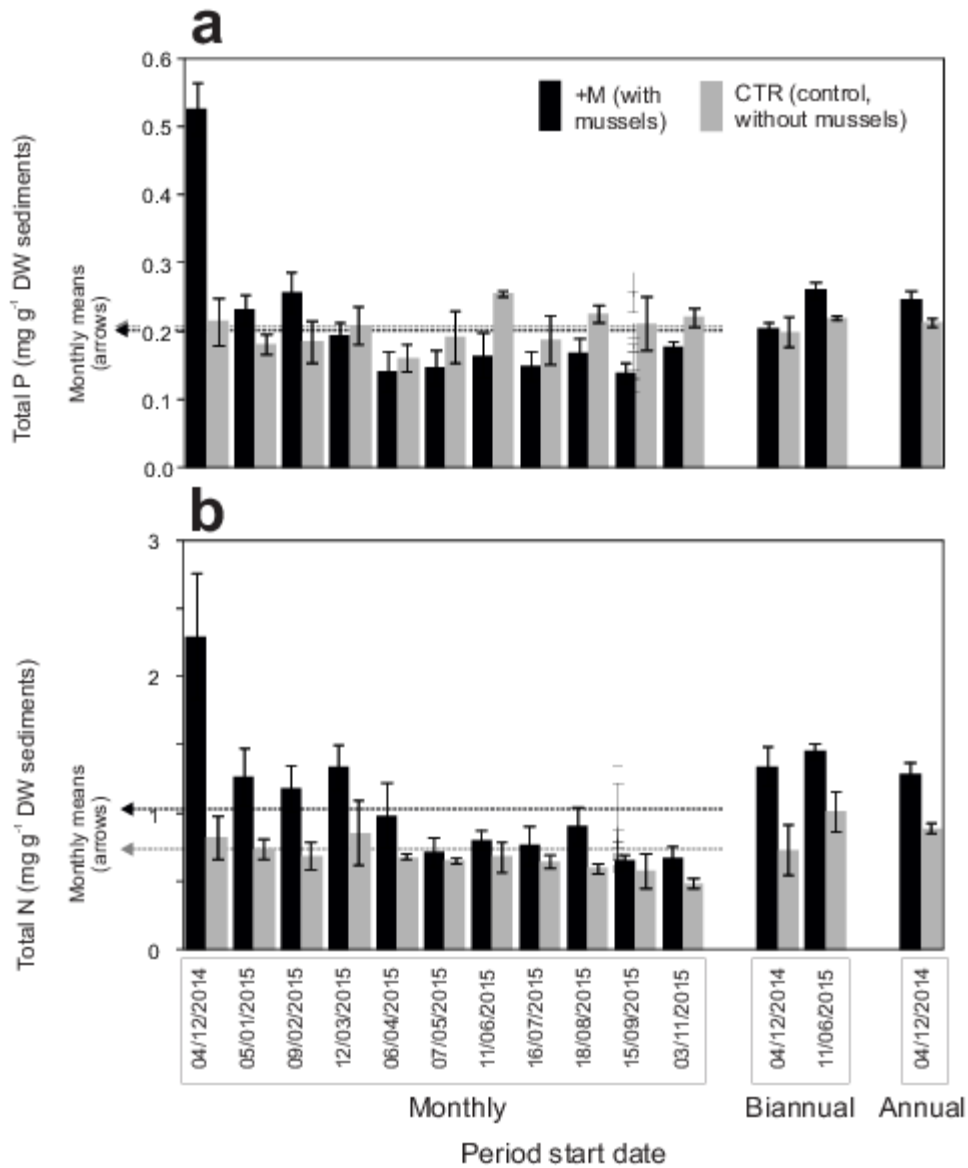


**Figure 5.** Relationships between the variables assessed in CTR vs. +M. Regression lines and R and P values are indicated for the significant ( $P < 0.05$ ) correlations. All datapoints and regression lines are based on actual values, correlation R and P are Pearson's (P) or Spearman's (S; when either variable did not comply with normality). a, b: linear; d: power. See Table S1 in the supporting information for data and units.



**Figure 6.** Mean and SD values for % organic matter (a-c), total P (d-f) and total N (g-i) in the cores retrieved from the two biannual and the annual deployments at 2 cm intervals. Each value is based on 2-6 replicates. Values with mussels (+M) are normalized to the average mussel biomass for all experimental units (5.2 g of mussel tissue dry weight). Notice that experiments with mussels accumulated more sediments, and therefore their cores are longer than those of the controls (CTR).

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**Figure 7.** Mean and SD values for total P (a), and total N (b) in sediments accumulated in the experimental units. Bimonthly and annual data are means of all 2 cm intervals in the cores obtained from the EU (Figure 6). Difference between CTR and +M means, total P:  $P=0.482$ ; total N:  $P=0.002$  (Mann-Whitney tests). See Table S1 in the supporting information for data and units.