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[noraceh@ceh.ac.uk](mailto:noraceh@ceh.ac.uk)

**The effects of increased flow and fine sediment on hyporheic invertebrates and nutrients in stream mesocosms.**

Iwan Jones\*, Ivor Gowns†, Amanda Arnold\*, Stephanie McCall‡ and Mike Bowes‡

\* *School of Biological and Chemical Sciences, Queen Mary University of London, London, E1 4NS, UK.*

† *Environmental and Rural Sciences, University of New England, Armidale, NSW, Australia 2351.*

‡ *Centre for Ecology and Hydrology, Maclean Building, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK.*

*Correspondence: Ivor Gowns, P.O. Box 68, Armidale, New South Wales, Australia, 2350. E-mail: [growsni@yahoo.com.au](mailto:growsni@yahoo.com.au)*

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*Keywords: Hyporheos, river regulation, invertebrates, colmation, sedimentation.*

## Summary

1. River regulation and altered land use form two common anthropogenic disturbances in rivers worldwide. Alteration of the stream bed, through processes such as siltation, or hydrology through river regulation, are likely to modify hyporheic processes or clog interstitial space and therefore impact both hyporheic invertebrates and nutrient dynamics.
2. We tested the separate and combined effects of increased flow and increased fine sediment on hyporheic water quality and invertebrates in flume mesocosms. Each mesocosm contained two bed sediment types; clean sediment in the upstream section and experimentally colmated (EC) sediment (10% by weight of fine sediment) in the downstream section. Two flow rates were initially established, a higher flow rate to create turbulent flow in six mesocosms and a lower flow rate to create a transitional flow between turbulent and laminar flows in the remaining six mesocosms. After 30 days invertebrates and physico-chemistry were sampled at three depths (5, 11 and 18 cm) and the flow in six of twelve mesocosms switched. The experiment was concluded after sampling invertebrates and physico-chemistry at day 70.
3. The addition of fine sediment to the mesocosm bed generally increased ammonium and decreased nitrate and soluble reactive phosphorus concentrations, decreased oxygen penetration and altered invertebrate assemblage structure. Increased flow rates generally lowered ammonium concentrations, increased soluble reactive phosphorus concentrations, increased oxygen penetration and altered invertebrate assemblage structure. Our hypothesis that higher flows would ameliorate any effects of added fine sediment was generally supported for oxygen penetration and nitrate concentration. However, we observed no differences in the interaction effects between flow regime and sediment types either on nutrient concentrations or invertebrate assemblage structure.
4. The rates of flow used in our mesocosms did not appear to reach the threshold required to remove fine sediment. It is generally recognised that river hyporheic restoration requires a set of objectives against which the outcomes can be measured yet this is often overlooked. Our research provides preliminary guidelines that small amounts of fine sediment can have deleterious ecological effects. However, further research is required to evaluate if lower

percentages of bed fine sediment result in ecological impairment and to determine what flow rates are required to ameliorate colmation impacts.

## **Introduction**

The hyporheic zone is the interface between aquifer and surface water and acts as habitat for organisms (Stubbington, Wood & Boulton, 2009; Nogaro et al. 2013) and a region of biogeochemical cycling (Mulholland et al., 2008; Pinay et al., 2009). The zone is temporally and spatially active with its boundaries changing with sediment structure and hydrological dynamics (Boulton & Stanley, 1995; Robertson & Wood 2010). Due to widespread anthropogenic influences, alteration of hydrological exchange occurs through processes such as increased fine sediment delivery from agricultural practices, or changes to hydrology through river regulation. Alterations to hydrology and sediment delivery may occur independently or concurrently affecting both nutrient dynamics and hyporheic (subsurface) dwelling invertebrates (Marmonier et al., 2012; Krause et al., 2011).

Increased sediment load, particularly deposition of fine sediment (i.e. inorganic and organic particles less than 2 mm in size) can lead to siltation, or colmation (Olsen, Matthaei & Townsend, 2010). Colmated sediments are characterized by reduced porosity and hydraulic connectivity which has significant implications for the efficiency of biogeochemical cycling and habitat conditions (Brunke 1999; Nogaro et al., 2010). Weakened mixing between the surface and hyporheic zones decreases oxygen exchange and promotes reducing conditions, thus altering the nutrient dynamics (Brunke & Gonser, 1997), by directly limiting oxygen (Baker, Dahm & Valett, 2000) or indirectly by changing bacterial communities (Findlay & Sobczak, 2000). Nutrient dynamics may be affected as anoxic conditions can decrease nitrate concentrations by denitrification (House & Denison, 1998; House, Leach & Armitage, 2001) and fine particles can sequester forms of phosphorus (Vervier et al., 2009). The amount and type of organic matter of fine articles can also affect nutrient dynamics (Stelzer et al 2014). Hyporheic invertebrates can play a role in maintaining porosity by feeding and burrowing through bioturbation (Danielopol, 1989; Nogaro et al. 2006). Boulton et al. (1997) suggested if habitat conditions deteriorate to a point to affect hyporheos, interstitial spaces can rapidly

clog, creating anoxic zones negatively impacting hyporheos assemblages. Although there is much research examining effects of colmation on hyporheic oxygen concentrations (e.g. Meyer et al., 2008) there is relatively little work determining the potential effects on nutrient dynamics and invertebrates.

The exchange of water between groundwater, hyporheic and surface layers is controlled in time by changes in discharge and in space by geomorphology (Fellows, Valett & Dahm, 2001; Kasahara and Wondzell, 2003). The exchange is complex but generally depends on the flow rate of the surface water (Hancock & Boulton, 2005). During low flow, limited surface water enters the groundwater ecotone and the main contribution to flow comes from deeper groundwater or lateral aquifers (Rassam et al. 2013). Conversely, at higher flows surface water predominantly downwells into the hyporheic zone, altering subsurface chemistry and residence time (Stanley & Boulton, 1995). There are strong associations between surface-hyporheic hydrologic exchange and hyporheos assemblages (Swan & Palmer, 2000; Olsen & Townsend, 2003). River regulation can affect hyporheic zones through weakened fluxes between the sediment and surface due to colmation and decreased hydrological exchange (Hancock, 2002). These patterns are evident in both large and small rivers indicating that river regulation is an important determinant of hyporheic assemblage structure.

Hydrologic factors are known to influence the distribution of biota and ecological processes in the surface of streams (Growth & Davies, 1994; Armitage & Cannan, 2000). However, much less is known about how these factors affect the hyporheic zone. Spates have been shown to influence the hyporheos, particularly when the bed structure is disturbed (e.g. Boulton, Valett & Fisher, 1992; Dole-Olivier & Marmonier, 1992). Although some workers have suggested that low flows are unlikely to influence hyporheic nutrients and invertebrates (Hancock & Boulton 2005; James, Dewson and Death 2008), the importance of flow in determining the exchange of water would suggest otherwise. Furthermore, as flow is an important parameter controlling the colmation of sediments there is considerable potential for any effect of flow on the hyporheic environment to be altered by fine sediment (Stubbington et al. 2011).

The relationship between flow and colmation of sediments is not straightforward. Whilst peak flows can be associated with the delivery of large quantities of fine sediment (Gibson, 2002), frequently occurring floods tend to winnow out fine sediments (Mürle, Ortlepp & Zahner, 2003). In simple terms, the behaviour of fine sediment within rivers is influenced by hydrologic conditions, the load of fine sediments entering rivers is largely dependent on landscape erosion within the catchment (Boulton et al., 1997), with both factors interacting to govern the rate of colmation. As drivers such as forestry and agricultural intensification are associated with changes to both flow and fine sediment load there is considerable advantage to understanding their separate and combined influence on hyporheic conditions. Despite the potential for flow and fine sediments to interact to govern physical and chemical hyporheic conditions, we lack experimental investigations of the combined effects of fine sediments and flow on hyporheos.

We present an experimental investigation where bed fine sediment and flow rates were manipulated and we examined their individual and joint effects on hyporheic nutrients and invertebrate communities. Our first two hypotheses were that differences in discharge and fine sediment bed loads would individually alter hyporheic physicochemical properties and invertebrates assemblages. In addition, we tested a third hypothesis that there would be an interaction between hydrology and siltation whereby higher flow rates would ameliorate the effects of increased levels of fine sediment.

## **Methods**

### *Study area*

The River Frome extends from the Dorset – Somerset border, to Poole Harbour. It has a catchment area of 414 km<sup>2</sup> (Marsh & Hannaford, 2008), which is mainly underlain by Cretaceous Chalk bedrock. A fuller description of the geology is given by Arnott, Hilton & Webb (2009). The land use is primarily agricultural (mainly grassland and cereals), with some watercress aquaculture. Dorchester is the only significant urban area in the catchment, with a population of 19,000 in 2013 (Office of National Statistics, 2014). For the period 1965 to 2005, the mean annual rainfall at East Stoke was

1020 mm, the mean annual discharge was  $6.38 \text{ m}^3 \text{ s}^{-1}$ , and the baseflow index was 0.84 (Marsh and Hannaford 2008). Median nutrient concentrations in the Frome River between 2009 and 2012 near the study area were as follows:  $0.04 \text{ mg L}^{-1}$  (range 0.013 to 0.182) for ammonium;  $0.07 \text{ mg L}^{-1}$  (0.023 to 0.250) for nitrite;  $26.85 \text{ mg L}^{-1}$  (15.80 to 37.37) for nitrate and;  $50 \text{ (30 to 190) } \mu\text{g L}^{-1}$  for soluble reactive phosphorus (Wessex Water, unpublished data).

### *Experimental design*

The experiment was carried out between May and August 2012 in twelve open air, flow-through flume mesocosms located at the Freshwater Biological Association's River Laboratory ( $50^{\circ}40'49'' \text{ N}$ ,  $2^{\circ}11'05'' \text{ W}$ ) in Dorset, U.K. Four blocks of mesocosms were sited adjacent to and fed from the Mill Stream, a side channel of the River Frome. Each block consisted of three aluminium linear flumes (0.33 m width, 12.4 m length and 0.30 m depth) positioned at approximately  $140^{\circ}$  to the riverbank and aligned side by side in an east-west direction.

Mesocosms were filled to a depth of 20 cm with sediment sourced from a local quarry to replicate the sediment-size distribution of the Mill Stream (volumetric proportions of particle sizes, 85% 11–25 mm, 5% 2–11 mm, 5% 0.35–2 mm, 5% 0–0.35 mm (Armitage, 1995; Ledger et al., 2008). To mimic internal colmation, river fine sediment, obtained from dredged river bed material, was added to sediment placed in the downstream 4 m stretch of each mesocosm. In these sections total fine sediment represented 10% of sediment weight. We chose 10% as ecological impairment potentially occurs when fine sediment reach this level (Kemp et al., 2011). Therefore, each mesocosm contained 2 bed sediment types; clean sediment in the upstream most 4 m section and experimentally colmated (EC) sediment in the downstream most 4 m section.

Unfiltered river water was delivered at the head of each block through an upstream inflow pipe (110 mm diameter) into a reservoir, approximately 2 m long, 1 m wide and 0.35 m deep. From the reservoir, water flowed by gravity over a low weir into the upstream section of each channel creating a localised impact in the first 100 mm of the upstream section in each mesocosm. Flow rates

in the individual mesocosms were controlled by adjusting the height of the weir. Invertebrate colonised the mesocosms drift from the Mill Stream (Harris, 2006).

Two flow rates were initially established in the twelve mesocosms. The higher flow rate (mean  $5.3 \times 10^{-3} \text{ m}^3 \text{ s}^{-1}$  range  $4.7 \times 10^{-3}$  to  $6.1 \times 10^{-3}$ ) was chosen to create turbulent flow (Reynolds number  $>2000$ ) and the lower flow rate ( $3.2 \times 10^{-4} \text{ m}^3 \text{ s}^{-1}$  range  $2.7 \times 10^{-3}$  to  $3.9 \times 10^{-3}$ ) was chosen to create a transitional flow between turbulent and laminar flows (Reynolds number between 500 and 2000). The higher and lower flow rates were assigned to each mesocosm alternately along the east-west direction. Flow was started in each mesocosm in May 2012. After 40 days, flow rates in six out of twelve of the mesocosms were switched; three of the higher flow flumes were altered to the low flow rate and three of the slower flow mesocosms were increased to the high flow rate. Therefore we had four flow scenarios, fast flow throughout the experiment (FF flow scenario), slow flow throughout (SS), fast flow for 40 days and then changed to slow flow (FS) and slow flow for 40 days changed to fast flow (SF). The experiment was concluded at day 70.

Prior to loading sediment into the mesocosms three replicate five litre sediment samples were randomly taken from clean and EC bed sediments. Bed sediment types were dried and sieved into the following size fractions:  $<0.125 \text{ mm}$ ,  $0.25 \text{ mm}$ ,  $0.5 \text{ mm}$ ,  $1.0 \text{ mm}$ ,  $2.0 \text{ mm}$ ,  $4.0 \text{ mm}$ ,  $8.0 \text{ mm}$  and  $16 \text{ mm}$  or greater size fractions. Percentage organic matter was calculated by subtracting the weight of sediment following loss on ignition by combustion at  $450 \text{ }^\circ\text{C}$  in a muffle furnace. At the end of the experiment to corroborate that sediment type had remained consistently different between the upstream and downstream of the mesocosms over the 70 days, the vertical distributions of fine sediment between bed sediment types were assessed by freeze coring. A  $12 \text{ mm}$  diameter copper pipe  $40 \text{ cm}$  in length with a pointed end was pushed into the bed sediment till it touched the bottom of the mesocosm. Liquid nitrogen was poured into the tube to freeze the surrounding water and sediment particles and the frozen material (core) removed from the surrounding sediment. The core was divided into three equal lengths, defrosted and the weight of fine sediment and organic content measured.

### *Sampling protocol*



Before the start of the experiment one cluster of sampling tubes were inserted into each of the upstream and downstream ends of each 4 m sediment section in each mesocosm. Each cluster comprised three sampling tubes approximately 20 cm apart and inserted to depths of 5, 11 and 18 cm. The sampling tubes were made from 12 mm diameter PVC pipe with four 5 mm diameter holes located on the vertical sides of the pipe 10 mm from the sealed bottom. A foam plug sealed the holes between sampling occasions. Invertebrates and water samples were collected from the sampling tubes 30 and 70 days after the commencement of flow within the channels. Invertebrate colonisation of the hyporheic environment can be rapid, within a matter of hours (Schmid-Araya 2000). However, as it was unknown how long the invertebrate assemblage would take to mimic a natural assemblage, we chose 30 days for the first sampling as this was similar to the 42 days Harris (2006) used for colonisation of the channels for benthic macroinvertebrates.

At sampling, the foam plug was pulled from the base of the sampling tube drawing water from the zone immediately adjacent to the 5 mm holes into the sampling tube. Water for nutrient analyses was collected by drawing 20 ml of sampling tube water through a 0.45 µm filter using a syringe fitted with a flexible hose. Water samples were frozen immediately for subsequent analysis. Soluble reactive phosphorus (SRP) concentrations were determined using the colorimetric methods of Murphy & Riley (1962). Ammonium,  $\text{NH}_4^+$ -N concentrations were determined colorimetrically using an indophenol blue method (Leeks et al., 1997). Nitrate and nitrite concentrations were determined by Ion Chromatography (Dionex DX500). Invertebrates were sampled by drawing 500 ml of water from the sampling tube, using a separate syringe fitted with a flexible hose. The water was then passed through a 250 µm sieve and the animals preserved in 4% formaldehyde. Invertebrates were identified to Family level, with the exception that chironomid larvae were taken to subfamily, and mites, nematodes and ostracods to Order.

Depth of oxygen penetration into the bed sediment was assessed using 6 mm diameter untreated softwood dowels, as described by Marmonier et al. (2004). Dowels were inserted vertically to the bottom of the mesocosms. Four such dowels were inserted at even spacing along each of the upstream and downstream 4 m sections of each mesocosm. Dowels were inserted at the start of the

experiment and removed following water quality and invertebrate sampling on day 30. The depth of oxygen penetration was assessed by measuring the length of each dowel that remained unstained below the bed surface. Fresh dowels were inserted on day 40, when flows were altered in six of the mesocosms, and removed and measured on day 70.

### *Data analysis*

Differences in the bed sediment particle size between the two sediment types at the start of the experiment were tested using permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) in the PERMANOVA+ for PRIMER software (Anderson, Gorley & Clarke 2008). PERMANOVA can be used to perform a main factors type analysis of variance (ANOVA), based on a matrix of similarities between sites. Significant relationships between the main factors (particle size and sediment type) and their interaction were tested using 9999 randomisations, based on Euclidean distances between samples. Similarly, differences in the bed sediments characteristics between sediment type and flow scenario at the end of the experiment were also tested with PERMANOVA. Differences in depth of oxygen penetration into the mesocosm bed between sampling occasions, flow scenarios and sediment types were tested using PERMANOVA, using Euclidean distances between samples.

We tested differences in physicochemical variables and invertebrate assemblage structure between sampling occasions, flow scenarios, depths and bed sediments using PERMANOVA. Position of samples in the mesocosms was incorporated as a covariate to account for any potential longitudinal effects. The main factors of interest to test our hypotheses were sediment type and flow scenario and the interaction between the two factors to test for any ameliorative effect of increased flow on experimental colmation. To compare among flow scenarios, we used planned contrasts to compare physicochemical variables and invertebrate assemblage of the fast-flow scenarios with the slow-flow scenarios at on the first sampling occasion (FF and FS with SS and SF) and second sampling occasion (FF and SF with SS and FS). In addition, planned contrast were used to compare within the fast-flow and slow-flow scenarios on both sampling occasions, i.e. FF with FS and SS with

SF on the first sampling occasion and, FF with SF and SS with FS on the second sampling occasion. Planned contrasts allowed us to examine pairs of flow scenarios in specific comparisons rather than examining all possible post-hoc pairwise comparisons.

Nutrient data and invertebrate abundance data were modified to using logarithmic and square root transformations, respectively, to minimise potential effects of skewed distributions. Invertebrate abundance data was range standardised prior to analysis. Euclidean distance was used as input for the PERMANOVA analysis of physicochemical data and Bray-Curtis distance was used to form similarity matrices for the invertebrate data. Significant relationships between the main factors and interactions were tested using 9999 randomisations. Patterns of differences in invertebrate assemblages identified by PERMANOVA were presented diagrammatically using non-metric multidimensional scaling (NMDS) using 50 randomised starts (Clarke, 1993). Similarity percentages (SIMPER) (Clarke, 1993) were used to identify invertebrate species contributing to differences in main factors identified by PERMANOVA.

## **Results**

### *Sediments*

At the start of the experiment, there was a significant difference in the percentage weight of fines between particle size categories (pseudo-F = 8.5,  $p = 0.007$ ) and bed sediment type (pseudo-F = 314,  $p = 0.0001$ ). The mean total percentage of fine particles was greater (9.8 % w/w) in EC sediment than clean sediment (6.2 % w/w) (Figure 1). The mean organic content of EC sediment was also greater (4.8% w/w) when compared with clean sediment (0.0 w/w).

At the end of the experiment, the distribution of fine sediment at different depths from the freeze cores corroborated that significant differences still existed between the two sediment types (interaction between depth and sediment type (pseudo-F = 13.2,  $p = 0.0001$ ). Percentage fine sediment increased with depth but at a greater rate in the EC sediment than the clean sediment (Figure 2). There was no significant difference in the distribution of fine sediment between flow scenarios (pseudo-F = 1.2,  $p > 0.05$ ). The distribution of percentage organic matter at different depths was significantly

different between the two sediment types (interaction between depth and sediment type (pseudo-F = 3.4,  $p = 0.027$ ). The percentage organic content decreased with depth for both sediment types but the rate of decline was greater in the EC sediment (Figure 2). There was no significant difference in the percentage organic content between flow scenarios or any interaction between flow scenarios and sediment types.

### Oxygen

Oxygen penetration was significantly different between all main factors including sediment type, flow scenarios and sampling occasions, explaining 15%, 2% and 14% of the variation, respectively (Table 1). However, there were significant pair-wise interactions between all three main factors (Table 1). Average oxygen penetration was generally greater for clean sediments than EC sediments (Figure 3). Between days 30 and 70 average oxygen penetration declined in all clean sediments irrespective of flow (Figure 3). Oxygen penetration remained the same in EC sediments when flows were not switched between days 30 and 70. However, where flows were switched from fast to slow, oxygen penetration was significantly reduced and when flows were switched from slow to fast oxygen penetration significantly increased (Figure 3, Table 1).

The interaction between sediment type and flow scenario can be explained because for all scenarios, except the FF scenario, the average oxygenation penetration was greater for the clean sediment compared with the EC sediment (Figure 3). For the FF scenario the average oxygen penetration was similar between sediment types (Figure 3). At the end of 30 days the average oxygen penetration was lower in the EC sediment compared with the clean sediment, but by day 70 oxygen penetration was similar (~ 100 mm) in both sediment types, giving a significant interaction between sediment type and time. The flow scenario by time interaction is explained by a decrease in average oxygen penetration from day 30 to 70 for all flow scenarios, except the SF scenario where the average penetration was similar between the two times (Figure 3). However, the fast flow scenarios had significantly greater oxygen penetration compared with the slow flow scenarios on both sampling

occasions and there was no significant difference within the fast or slow flow scenarios at either time (Table 1).

### Nutrients

Median concentrations of nutrients 5 cm below the bed in the mesocosms during the experiment were 0.06 mg L<sup>-1</sup> (range 0.003 to 4.375 mg L<sup>-1</sup>) for ammonium, 0.02 mg L<sup>-1</sup> (< 0.01 to 1.00 mg L<sup>-1</sup>) for nitrite, 20.88 mg L<sup>-1</sup> (<0.1 to 28.25 mg L<sup>-1</sup>) for nitrate and 23.0 µg L<sup>-1</sup> (1.0 to 129 µg L<sup>-1</sup>) for SRP. These values are similar to the concentrations observed in the River Frome (see study area description). Sediment type and flow scenario significantly influenced the concentration of ammonium and nitrate and only flow scenario influenced SRP and explained between 5% and 17% of the total variation (Table 1). However, the main factors also significantly interacted with depth and sampling occasion. None of the main factors influenced nitrate (Table 1). The interaction between flow scenario and sediment type was significant for ammonium and nitrate but explained 5% or less of the total variation.

The change in the concentration of ammonium with depth was significantly influenced by sediment type (Table 1). Pair-wise comparisons indicated that ammonium concentrations in the shallow layer were not significantly different between sediment types, but were significantly higher in the middle and deep sections of the EC sediment (Figure 4). The concentration of ammonium differed significantly between flow scenarios and was influenced by sampling occasion (Table 1). The planned contrasts indicated that on both sampling occasions there were significant differences between the fast and slow flow scenarios (Table 1). The fast flow scenarios had significantly higher concentrations of ammonium than the slow flows on both occasions (Figure 4).

The greatest source of variation for the concentration of nitrate was substrate type, explaining 23% of the total variance (Table 1). On average the concentration of nitrate was greater in the clean versus the EC sediment (Figure 4). There was no significant difference in the concentration of nitrate between the four flow scenarios on the first sampling occasion. However, on day 70 the concentration of nitrate was significantly lower in the EC sediment for three flow scenarios, with the exception of

the SF scenario (Figure 4), potentially explaining the significant three-way interaction between sampling occasion, sediment type and flow scenario (Table 1).

The mean concentration of SRP was significantly lower in the EC sediment than the clean sediment (Table 1, Figure 4). However, there were also significant effects of depth, flow scenario and sampling occasion on SRP concentration and significant interactions between some of these factors (Table 1). Pair-wise tests indicated that on the first sampling occasion the mean concentration of SRP on the bottom of the flumes was significantly lower than either the middle or shallow layers. However, on day 70 there was no significant difference between the different depths. Pair-wise tests indicated that the mean concentration of SRP was significantly lower in the SF flow scenario compared with the other scenarios, potentially explaining the significant main factor flow scenario.

### Invertebrates

A total of 5,183 invertebrates from 29 taxa were identified from the 288 samples. The most common taxon found was Cyclopoid copepods comprising 58% of the total abundance, followed by Chydoridae (15%), Gammaridae (7%), Ostracoda (4%), Tanytarsini chironomids (4%), Oribatida (1%) and Ephemerae (1%). The remaining 22 taxa each comprised less than one percent of the total abundance.

Each of the main factors significantly influenced invertebrate assemblage structure (Table 1). Five of the six two-way interactions were significant, however, only the interaction between flow scenario and sampling occasion explained greater than 2% of the total variation (Table 1). Pair-wise tests of invertebrate assemblage structure indicated the bottom two sediment layers differed significantly from the shallow layer but not from each other (Table S1). The interaction between depth and the other main factors was not significant explained less than one percent of the variation indicating that depth differences were generally maintained through time and between sediment types and flow scenarios (Table 1). SIMPER analysis indicated that Oligochaeta, Ephemerae and Tanytarsini were more abundant in the shallow layer compared with the two deep layers. In contrast,

four taxa, including Oribatida, Ostracoda, Gammaridae and Cyclopoida were more abundant in the deep layers (Table S2).

The differences in the invertebrate assemblages between sediment types explained 5% of the total variation (Table 1). SIMPER analysis indicated that seven taxa contributed 6% or more to the separation of assemblages between sediment types: six taxa including, Oligochaeta, Cyclopoida, Tanytarsini and Ephemeraeidae were more abundant in the clean sediment, whereas, Ostracoda, Oribatida, and Gammaridae were more common in EC sediment (Table S3).

On day 30, the invertebrate assemblages in the fast (FF and FS) flow scenarios were not significantly different to each other but were significantly different to those from the slow (SS and SF) flow scenarios (Table 1). This result is shown in the nMDS where the fast flow scenarios and slow flow scenarios, respectively, occupy similar positions in the ordination space, indicating that the invertebrate assemblages are similar to each other (Figure 5). Similarly on day 70, the fast (FF and SF) scenarios were not significantly different to each other but were significantly different to the slow (SS and FS) flow scenarios, explaining the interaction between sampling occasion and flow scenario (Table 1). These results suggest that the invertebrate assemblages responded to flow within the mesocosms; switching the flow from fast to slow or from slow to fast at day 40 resulted in the faunal assemblage changing to become the same as the slow controls (SS) or fast controls (FF), respectively at day 70 (Figure 5). The invertebrate taxa that contributed more than 6% to the significant differences between flow scenarios on day 30 using SIMPER analysis included, Oligochaeta, Gammaridae, Tanytarsini, Orthocladinae, Ostracoda and Ephemeraeidae, which were all more abundant in the faster flowing mesocosms (Figure 5, Table S4). Three taxa more abundant in the fast flows (Oligochaeta, Ephemeraeidae, Oribatida) and two taxa less abundant (Ostracoda and Sphaeriidae), contributed more than 6% to significant differences between flow scenarios on day 70 (Figure 5, Table S4).

## **Discussion**

We have demonstrated that the addition of fine sediment and the differences in flow affect hyporheic oxygen penetration, nutrients and invertebrates, supporting our first two hypotheses. However, the

specific effects may be modified through time and with depth. Our third hypothesis that higher flows would ameliorate any effects of added fine sediment was generally supported for oxygen penetration. However, we observed no major interaction effects between flow regime and sediment types either on nutrient concentrations or invertebrate assemblage structure. These results are in contrast with the assertion that increased flow can reduce the effects of sedimentation through winnowing (Mürle, Ortlepp & Zahner, 2003). It is possible that the differences between the turbulent and transitional flow regimes (and their alteration) in our mesocosms did not reach a requisite threshold to create enough shear stress to remove fine sediment or alter sediment dynamics. Boulton, Harvey & Proctor (2004) observed only limited change in sediment dynamics within the hyporheic zone following an artificial spate that created a three-fold increase (at least) in discharge. Further manipulative experiments are required to determine the flow rates required to mitigate the impacts of excess fine sediments in stream beds. Such experiments would assist in the management of sedimentation of rivers. Gravel cleaning operations are often conducted as a management tool to restore the quality of gravel stream beds sufficient to make them suitable for salmonid spawning (Shackle, Hughes & Lewis, 1999; Merz et al., 2004). However, without addressing the sources and retention of fine sediment, the indications are that such gravel cleaning approaches only provide a temporary improvement in habitat quality and therefore the spawning success of salmonids (Kasahara et al. 2009; Pulg et al., 2013).

Although the substrate had been well mixed before adding it to the mesocosms, by the end of the experiment most of the fine sediment, in both sediment types, had settled to the bottom of the mesocosms. There was also an increase in the amount of fine sediment in the substrate, presumably as a consequence of deposition from the inflowing water; by the end of the experiment fine sediment, comprised 18% by weight in the experimentally colmated reaches. Oxygen penetration was lower in the EC sediment at day 30 but was similar in both sediment types at the end of the experiment. The concentration of ammonium was generally higher in the EC sediment compared with the clean sediment, the concentrations of nitrite similar and nitrate lower. Nitrite is produced as an intermediate in both nitrification and denitrification. The similarity of free nitrite in the both sediment types is, therefore, potentially a result of the complex balance between diffusive transport, aerobic ammonium



oxidation, and anaerobic nitrate reduction. The addition of fine sediment caused an increase in anoxic conditions (Figure 4) that altered the nutrient dynamics in favour of denitrification. These results are supported by Meyer et al. (2005) who demonstrated that although nitrite accumulated from both aerobic ammonium oxidation and anaerobic nitrate reduction, the nitrate reduction rather than ammonium oxidation was the major direct source of nitrite in the anaerobic sediment layer. Another potential pathway of a lower concentration of ammonium in the clean substrate is conversion to dinitrogen gas via anaerobic ammonium oxidation (anammox). The clean substrate had a higher percentage organic content compared with the EC sediments and anammox activity has been positively correlated with concentration of the organic carbon content of estuarine sediments (Nicholls & Trimmer 2009). However, further research is required to determine the relative contribution of anammox and denitrification activity in freshwater sediments and how this may be modulated by organic carbon.

The mean concentration of SRP was generally lower in the EC sediment. Phosphorus dynamics in rivers are controlled by biotic (e.g. assimilation by both primary producers and decomposers) and abiotic factors (e.g. sorption to and release from suspended and benthic particle surfaces) (Reddy et al., 1999; Mainstone & Parr 2002). Abiotic control on phosphorus in streams and rivers is often driven by particle size (House and Denison 2000), with small particles being particularly effective in sequestering soluble forms (Klotz, 1988; He et al., 1995). Although there may be some biotic influence which caused the lower SRP concentrations in the fine sediment-added sediment, it is highly likely that the increase in the percentage fine sediment resulted in an increase in the sorption potential of the EC sediment, thereby reducing the SRP concentrations in the pore water.

Several studies have suggested an impact of fine sediment pollution on hyporheic invertebrates (Descloux, Datry & Marmonier 2013; Mathers et al., 2014). However, the minimum amount of fine sediment required to cause a detrimental effect is often not reported. We have demonstrated that an increase of 3.6% fine sediment by weight (the difference between the fine sediment in the clean and EC sediments) is enough to alter invertebrate assemblage structure. However, the effect explained 5% of the overall variation in the invertebrate assemblage structure.

Further experimentation is required to determine whether lesser colmation rates will impact hyporheic invertebrates. In contrast to our findings, Pacioglu, Shaw & Robertson (2012) observed that of 32 taxa in the hyporheic assemblage only abundances of Oligochaeta differed between colmated and clean sediments. Fine sediment pollution can affect invertebrates directly through altered water quality (Bretschko, 1994), loss of microhabitat (Rae, 1987), loss of access to food resources (Lenat, Penrose & Eagleson 1981) and the damage to respiratory systems of individuals (Lemly, 1982) or indirectly by changing trophic sources (Quinn et al., 1992). Just as the relationship between benthic invertebrates and fine sediments is poorly understood (Jones et al., 2011), the conditions under which excess fine sediment causes detriment to the hyporheos, and the mechanisms that cause such effects, require further study.

We have demonstrated that hyporheic invertebrates responded to differences in the surface flow conditions and that a change in assemblage structure was evident after 30 days. Altering the surface flows resulted in a rapid change to the hyporheic invertebrate assemblage structure. These results are supported by previous studies which demonstrate hyporheic invertebrate assemblages may respond to droughts and floods and to upwelling and downwelling riffle sections (e.g. Stubbington et al. 2011). The response of the hyporheos to localised changes in surface flows suggests that their hydrological relationships may be more sensitive than originally thought and, therefore, we advocate the incorporation of measures of near-bed hydraulics into future studies.

In conclusion, we have demonstrated an increase in the proportion of bed fine sediment to 10% by weight decreases oxygen levels, increases ammonium concentrations, and alters invertebrate assemblage structure in the hyporheic zone. Different flow rates also affected these attributes. The rates of flow used in our mesocosms did not appear to reach the threshold required to remove fine sediment or alter sediment dynamics. It is generally recognised that river hyporheic restoration requires a set of objectives against which the outcomes can be measured (England et al., 2008) yet this is often overlooked. This may in part be because there are very few guidelines to help support the decision making processes in terms of the physical aspects of river restoration or linking these to chemical, hydrological or biological benefits. Further research is therefore required to evaluate if

lower percentages of bed fine sediment result in ecological impairment and to determine what flow rates are required to ameliorate sedimentation impacts.

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Table 1. Effect of experimental treatments on oxygen penetration, nutrient concentrations and invertebrate assemblage structure. Pseudo-F values, probability levels and percentage variation given for each source of variance in PERMANOVA models and for the planned contrasts. Significant sources of variation are shown where relevant.

Source of Variance	d.f.	Oxygen penetration		Ammonium		Nitrite		Nitrate		Soluble Reactive Phosphorus (SRP)		Assemblage Structure	
		Pseudo F	%	Pseudo F	%	Pseudo F	%	Pseudo F	%	Pseudo F	%	Pseudo F	%
Position (Po) – Covariate	1	NA		ns		ns		ns		ns		4.8***	4
Sediment type (ST)	1	81.7***	15	9.6**	17	ns		16.9***	23	ns		3.5***	5
Flow scenario (F)	3	5.1**	2	6.8***	5	ns		11.4***	6	5.8***	6	5.0***	4
Depth (D)	2	NA		3.3*	1	ns		ns		3.3*	2	4.0***	2
Sampling occasion (SO)	1	74.7***	14	11.4***	4	ns		10.6***	3	8.2**	5	42.3***	18
ST x F	3	3.3*	2	3.4*	4	ns		5.1**	5	ns		1.5*	1
ST x D	2	NA		5.9**	6	ns		4.3*	<1	5.5**	9	ns	
ST x SO	1	96.0***	36	ns		ns		8.3**	4	ns		3.8***	2
F x D	6	NA		ns		ns		ns		ns		1.3*	<1
F x SO	3	10.6***	7	6.6***	9	ns		14.2***	15	ns		4.2***	6
SO x D	2	NA		ns		ns		ns		4.0*	1	ns	
SO x ST x F	3	4.5**	5	2.8*	4	ns		5.0**	9	ns		ns	
Planned contrasts													
Time 1													
(FF,FS) vs (SS,SF)	1	41.4***		5.4*		ns		3.5*		ns		11.2***	
(SS) v (SF)	1	ns		ns		ns		ns		ns		ns	
(FF) v (FS)	1	ns		ns		ns		ns		ns		ns	
Time 2													
(FF,SF) vs (SS,FS)	1	11.5***		24.5***		ns		38.1***		5.3*		10.7***	
(SS) v (FS)	1	ns		ns		ns		4.7*		ns		ns	
(FF) v (SF)	1	ns		ns		ns		3.5*		10.7***		2.2*	

NA- not applicable, ns – not significant, \*\*\* -  $p < 0.001$ , \*\* -  $p < 0.01$ , \* -  $p < 0.05$ .

## Supporting Information

Table S1. Pairwise comparisons of invertebrate assemblages from different depths in the mesocosms using PERMANOVA.

Depth	T - value	Probability
Bottom vs Middle	1.26	0.0998
Bottom vs Shallow	2.40	0.0001
Middle vs Shallow	1.89	0.0003

Table S2. SIMPER results of macroinvertebrate taxa contributing to differences (Bray-Curtis distance) between depths.

Taxon	Average abundance in bottom and middle samples	Average abundance in shallow samples	Consistency Ratio	Contribution (%)
Oligochaeta	22.7	31.3	1.11	9.1
Ephemeroidea (Ephemeroptera)	11.0	26.0	0.82	7.9
Tanytarsini (Diptera)	13.3	23.1	0.88	7.3
Oribatida	20.6	10.0	0.76	6.8
Ostracoda	21.8	12.8	0.97	6.5
Gammaridae (Amphipoda)	19.6	14.7	1.05	6.0
Cyclopoida	33.4	29.0	1.11	6.0

Table S3. SIMPER results of macroinvertebrate taxa contributing to differences between clean and experimentally colmated sediments.

Taxon	Average abundance in clean sediment	Average abundance in experimentally colmated sediment	Consistency Ratio	Contribution (%)
Oligochaeta	21.0	16.5	0.83	11.8
Cyclopoida	24.7	21.3	0.93	9.0
Ostracoda	9.8	14.6	0.79	8.1
Tanytarsini (Diptera)	12.0	9.2	0.67	7.8
Oribatida	10.5	10.6	0.59	7.4
Gammaridae (Amphipoda)	10.6	10.9	0.82	7.2
Ephemeridae (Ephemeroptera)	10.3	6.9	0.51	6.0

Table S4. SIMPER results of macroinvertebrate taxa contributing to differences (Bray-Curtis distance) between fast and slow flows.

Taxon	Average abundance in fast flow	Average abundance in slow flow	Consistency Ratio	Contribution (%)
Time 1				
Oligochaeta	37.5	9.6	0.99	10.6
Gammaridae (Amphipoda)	30.3	11.3	0.90	8.7
Tanytarsini (Diptera)	32.3	16.6	0.96	8.7
Orthocladiinae (Diptera)	29.9	1.0	0.96	7.9
Ostracoda	19.8	11.7	0.82	6.7
Ephemeraeidae (Ephemeroptera)	21.6	2.7	0.63	6.1
Time 2				
Oligochaeta	47.6	19.6	1.34	10.1
Ephemeraeidae (Ephemeroptera)	36.9	2.7	1.05	9.6
Oribatida	29.3	28.6	1.03	8.3
Ostracoda	28.4	30.7	1.17	6.5
Sphaeriidae (Veneroidea)	14.9	15.4	0.74	6.2

## Figure captions

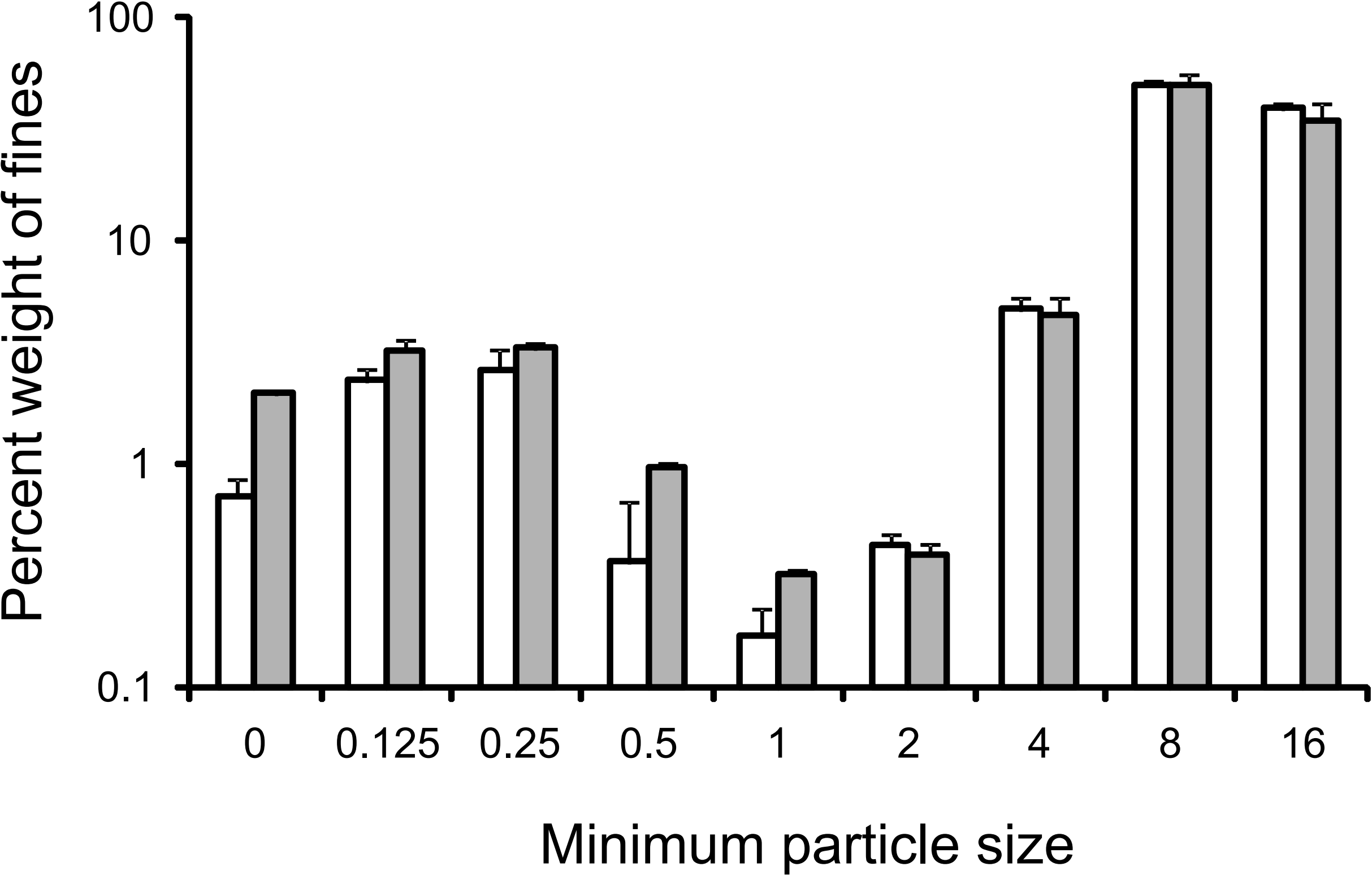
Figure 1. Mean ( $\pm 1$  S.E.) percentage weight of bed sediment particles among sizes classes for sediment loaded into mesocosms at Day 0. White columns indicate clean sediment and grey columns experimentally colmated sediment.

Figure 2. a) Mean ( $\pm 1$  S.E.) percentage weight of fine sediment (particles  $< 2$ mm) and b) percentage organic content in the shallow (0-6 cm), middle (6 – 12 cm) and bottom (12 – 18 cm) layers of bed sediment in mesocosms at Day 70. White columns indicate clean sediment and grey columns experimentally colmated sediment.

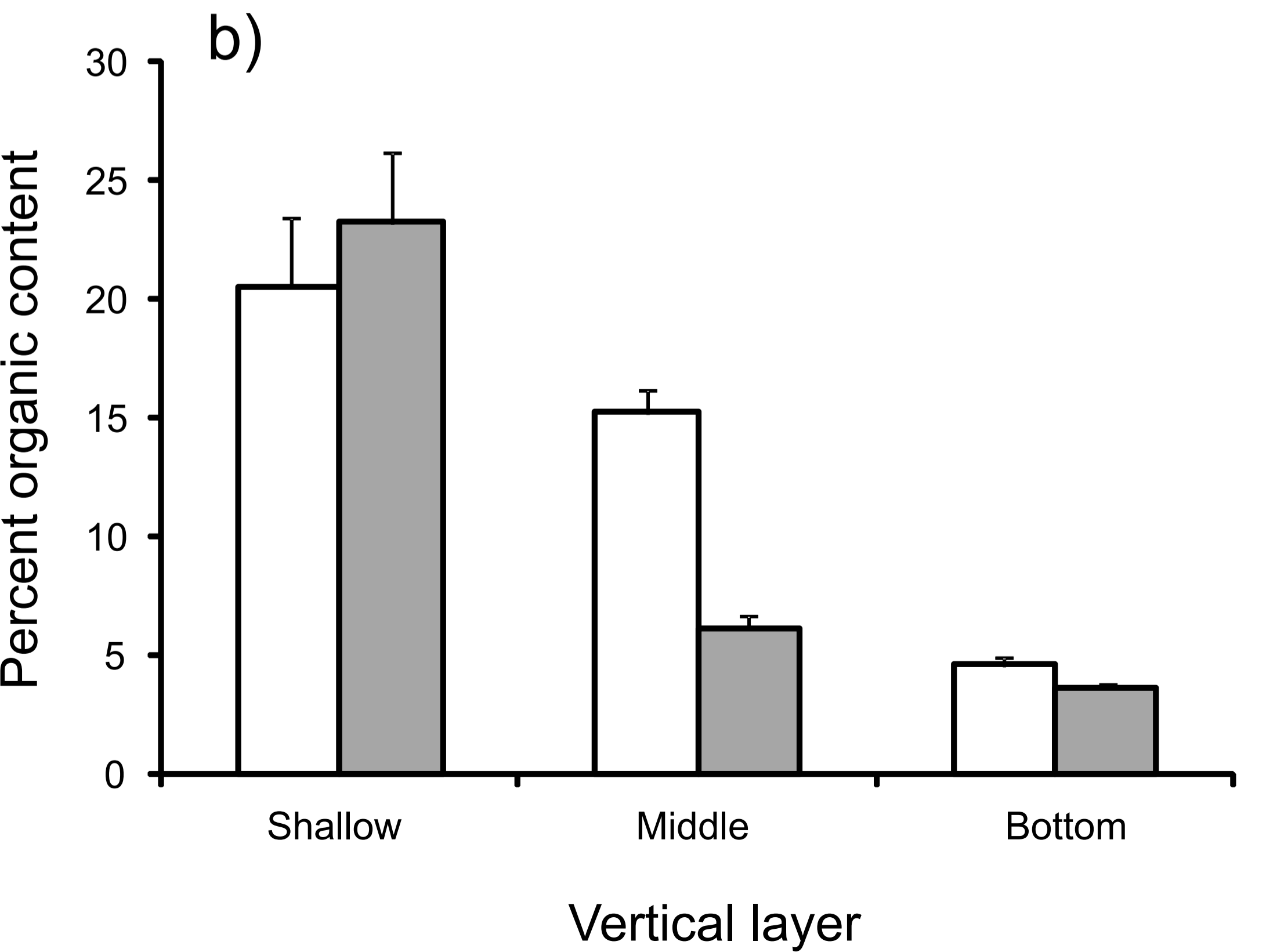
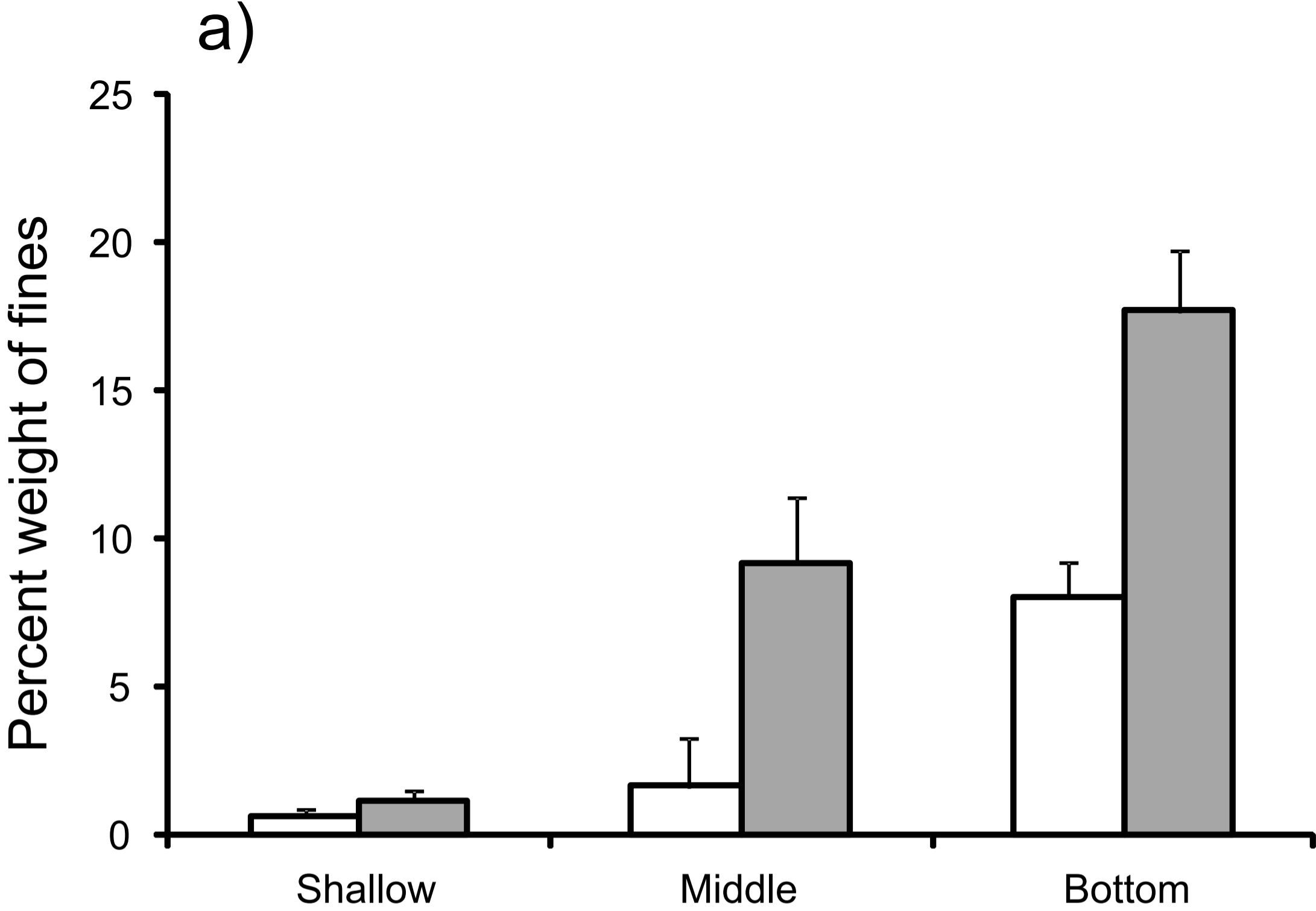
Figure 3. Mean ( $\pm 1$  S.E.) depth of oxygen penetration at day 30 and day 70 among flow scenarios, times and sediment types. White columns indicate clean sediment and grey columns experimentally colmated sediment.

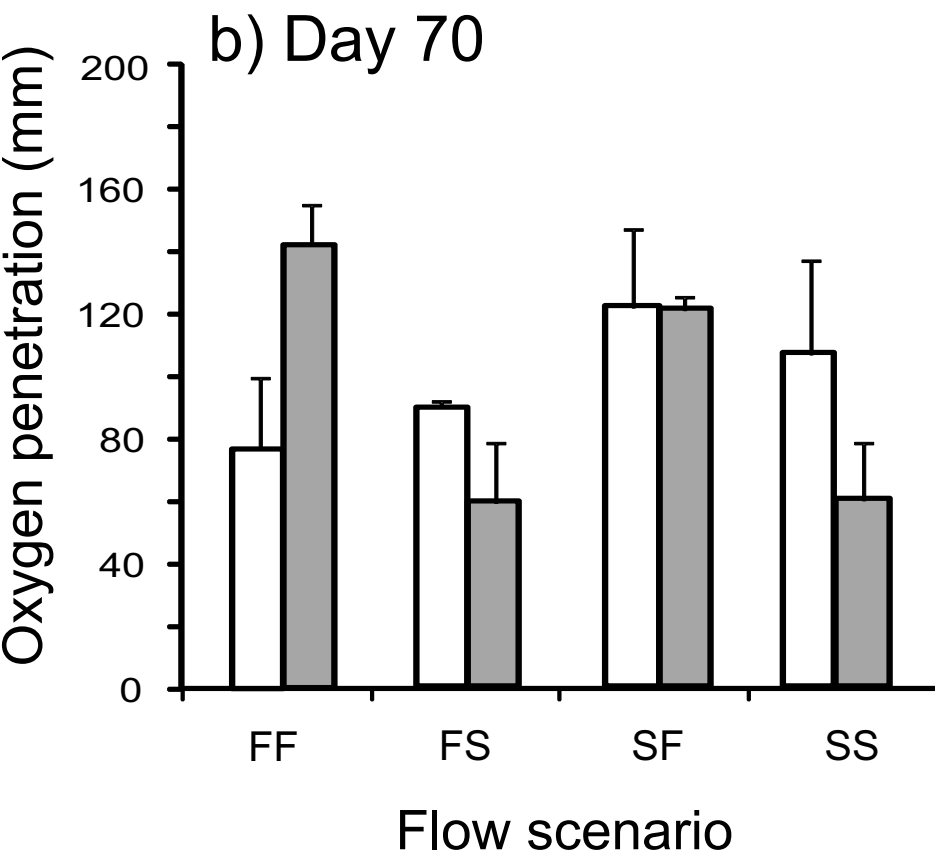
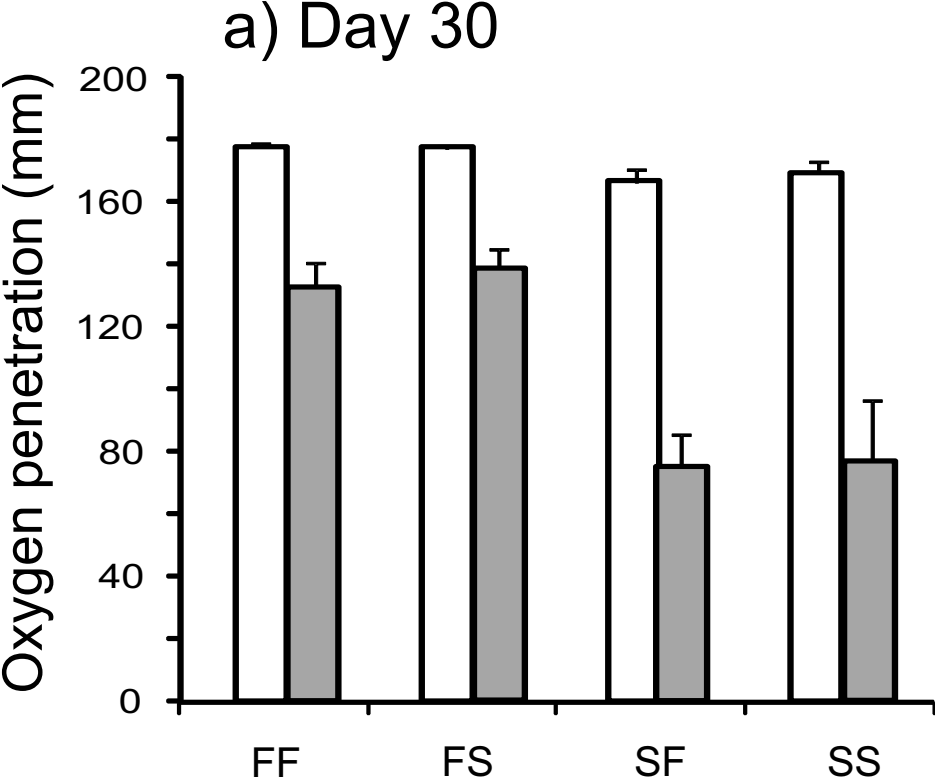
Figure 4. Mean ( $\pm 1$  S.E.) concentration of four nutrients at Day 30 and Day 70 in shallow (S), mid (M) and bottom (B) depth layers in clean sediment (white columns) and experimentally colmated sediment (grey columns) for four flow scenarios.

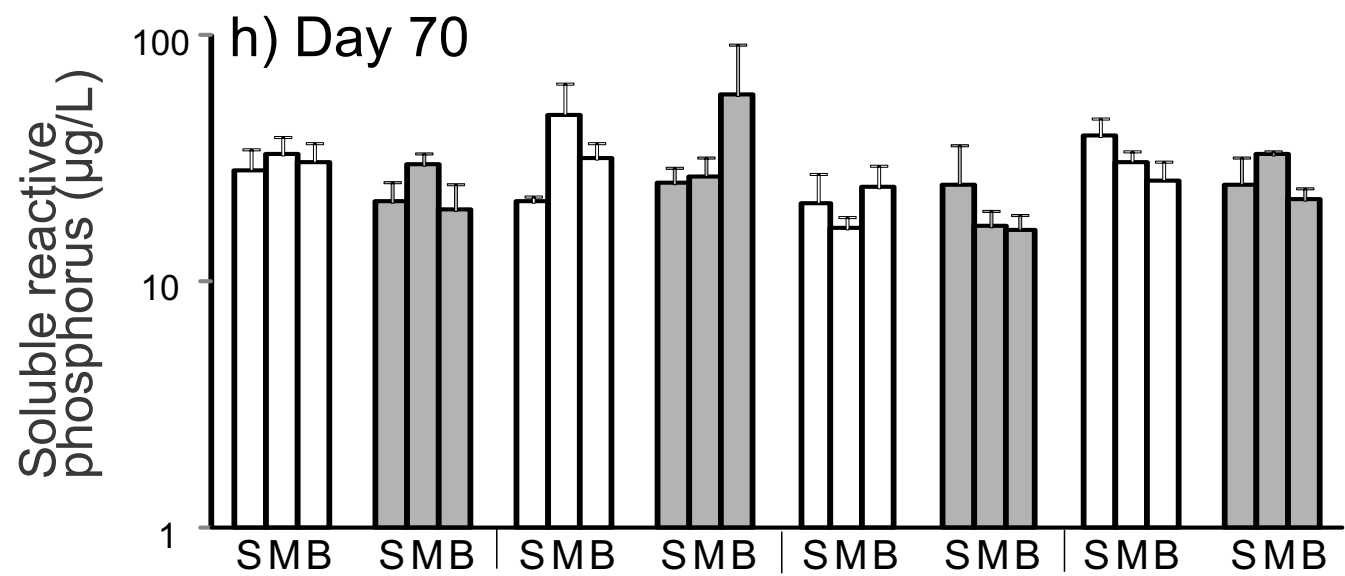
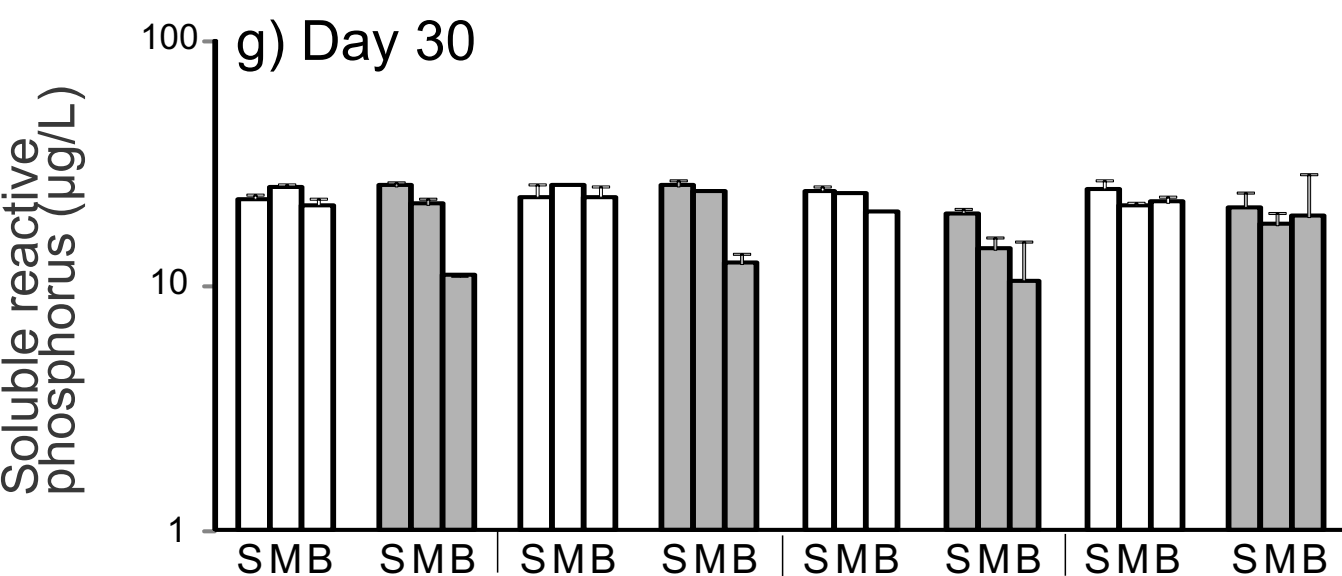
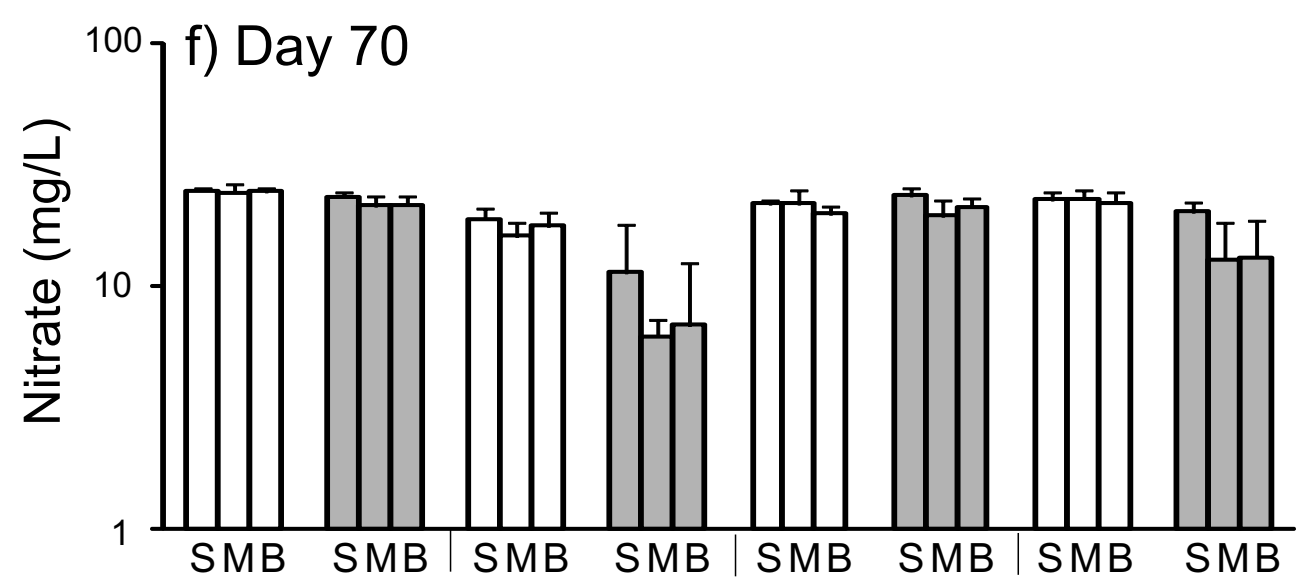
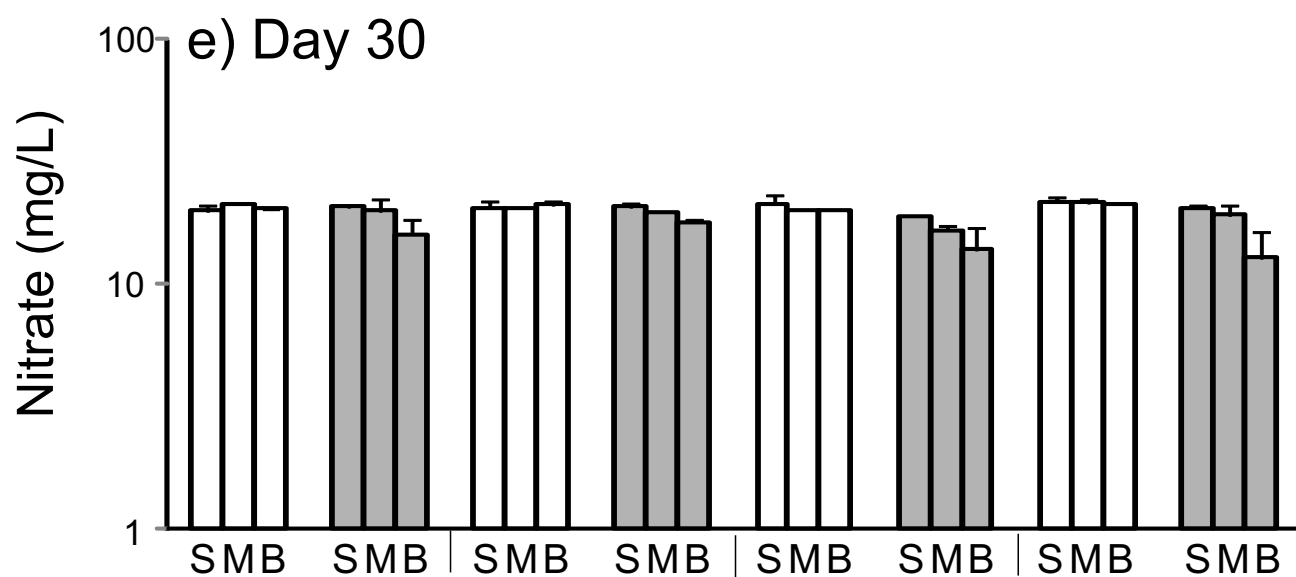
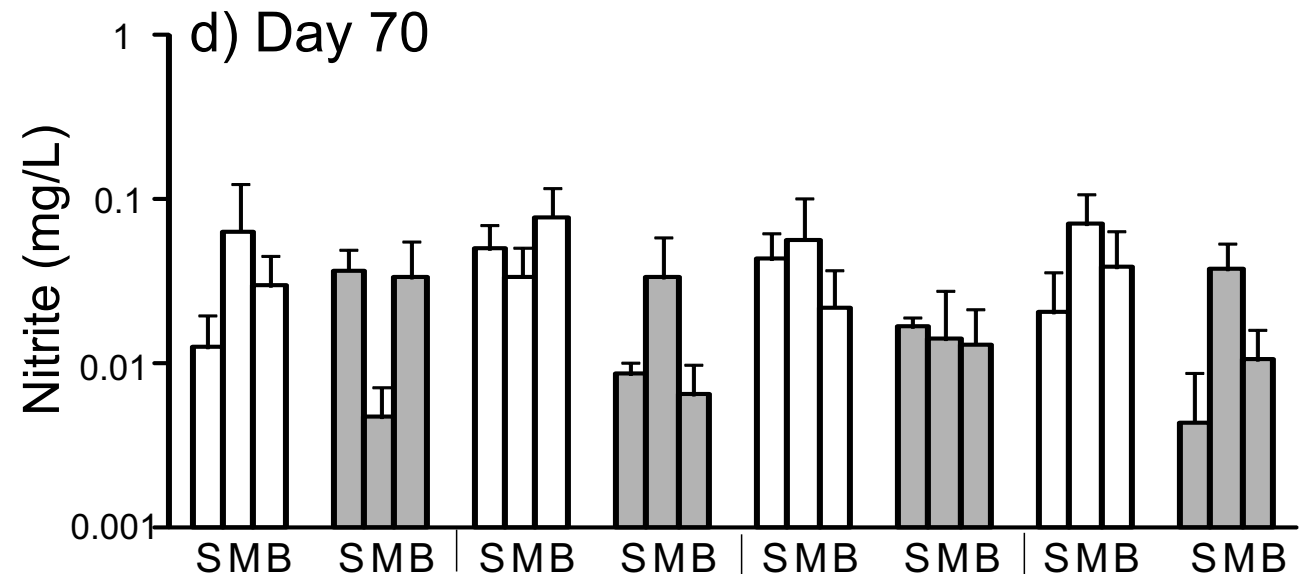
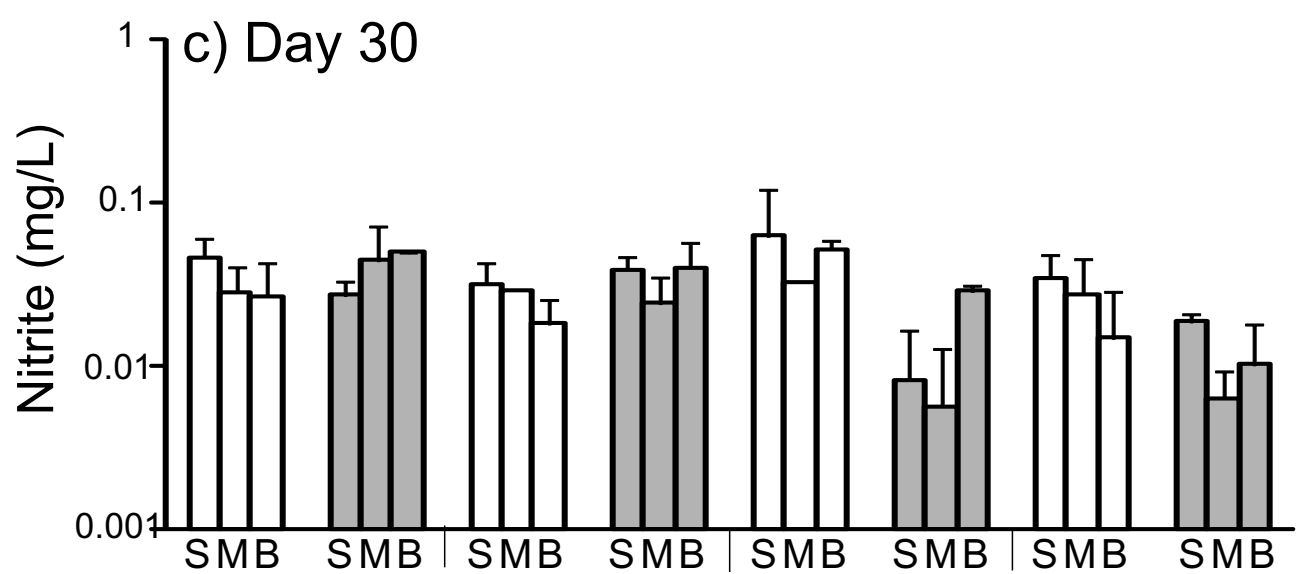
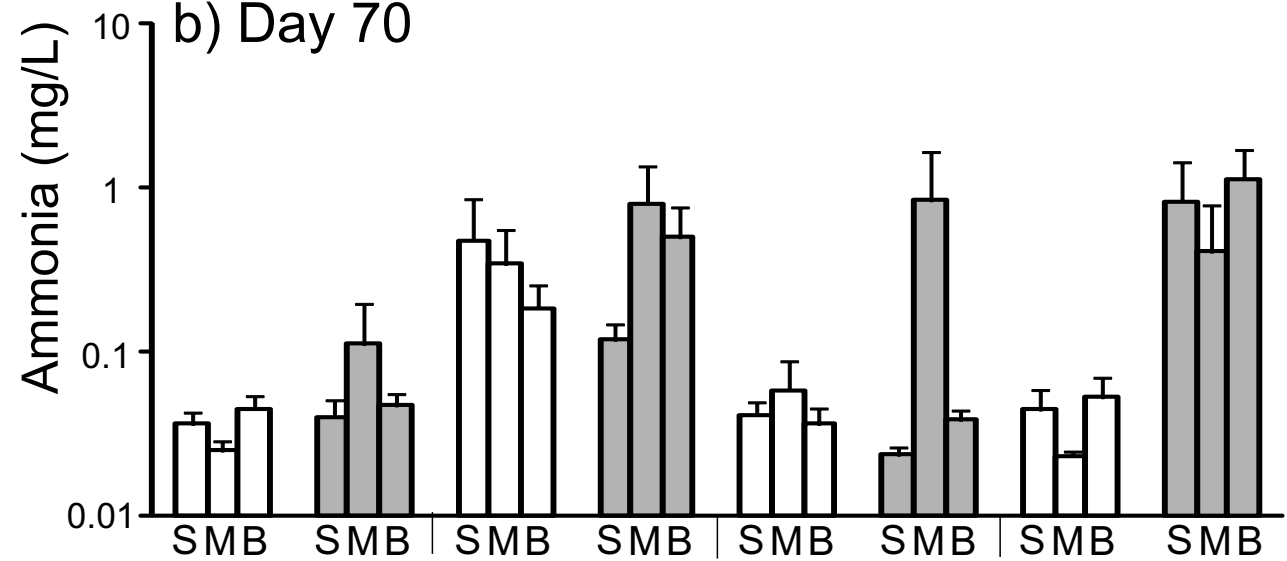
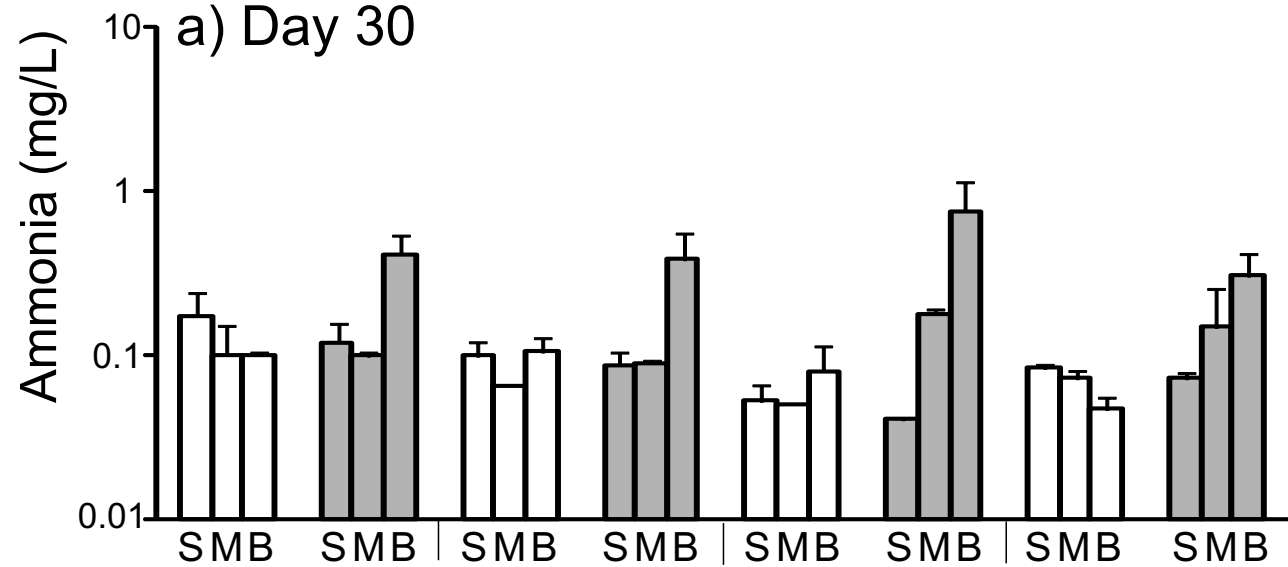
Figure 5. Ordination of centroids of samples from FF (stars), FS (squares), SS (triangles) and SF (circles) flow scenarios on day 30 (white symbols) and day 70 (black symbols). For definition of abbreviations see text. Arrows indicate direction of increased abundance of taxa.









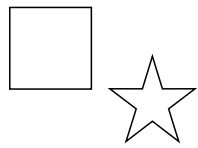


Flow scenario

Flow scenario



Ephemeraeidae  
Gammaridae  
Oligochaeta  
Orthoclaadiinae  
Ostracoda  
Tanytarsini



Ephemeraeidae  
Oligochaeta  
Oribatida  
Ostracoda  
Sphaeriidae

