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The hyporheic zone as an invertebrate refuge during a fine sediment disturbance event

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Abstract

Subsurface sediments offer an important refuge that support the survival and persistence of river invertebrates during adverse surface conditions. Access to refuges for invertebrates varies with differing hydrological and substrate characteristics, especially the proportion of fine sediment. This study examines whether substrate characteristics influence the use of the hyporheic zone as an invertebrate refuge during a fine sediment disturbance event. We used 12 outdoor stream mesocosms to examine the vertical migration of benthic and hyporheic invertebrates to fine sediment loading. Each mesocosm was filled with coarse or experimentally colmated sediments. After 69 days, a fine sediment pulse of three varying fine sediment concentrations were added to the stream mesocosms. Both before and after the fine sediment pulse, a distinct gradient in the abundance and richness of hyporheic invertebrates was apparent with depth. However, hyporheic abundance and taxonomic richness decreased at 5 cm and increased at 18 cm during fine sediment loading, indicating vertical migration of invertebrates from the benthic to hyporheic zone. Our study provides support for the hyporheic zone as a refuge for benthic invertebrates during fine sediment disturbance events. We also found evidence that movement pathways within subsurface sediments were still accessible and permitted bidirectional migration of individuals between the benthic and hyporheic zone in the coarse and colmated sediments during fine sediment loading. Understanding how increased fine sediment deposition affects streambed porosity will be increasingly important with ongoing climate change and anthropogenic sedimentation.

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1 INTRODUCTION

In river ecosystems, benthic and hyporheic sediments provide valuable habitat and refuges for aquatic biota during negative surface events (Williams & Hynes, 1974, Stubbington, 2012; Vadher et al., 2015). Vertical movement into subsurface sediments allows mobile invertebrates to persist through disturbance events in situ. Refuge habitat has an important ecological role in promoting population and community recovery (i.e., resilience) after a disturbance event (Gjerløv et al., 2003; Vadher et al., 2015; Hill & Milner, 2018). Previous research has examined the use of the hyporheic zone as a refuge during flood events (Holomuzki & Biggs, 2000; Boulton et al., 2004), surface freezing (Orghidan, 1959), increasing water temperatures and intraspecific competition (Evans & Petts, 1997; Wood et al., 2010; Vander Vorste et al., 2017), streambed drying (Vadher et al., 2015; Vadher et al., 2018) and low flows (James et al., 2008; Stubbington et al., 2011). However, evidence identifying the hyporheic zone as a refuge is equivocal (Dole-Olivier, 2011; Stubbington, 2012), with some studies finding no increased vertical migration into subsurface sediments (e.g., Olsen & Townsend, 2005; James et al., 2008; Young et al., 2011), whilst other studies have reported higher abundances in subsurface sediments after a disturbance (e.g., Vadher et al., 2015; Mathers et al., 2017; Mathers et al., 2019).

Sedimentation and clogging of benthic and hyporheic zone substrates are increasingly recognised as a key threat to the ecological integrity of riverine ecosystems globally (Mathers et al., 2017). Excess fine sediment is not delivered to rivers in a uniform manner, rather in pulsed events typically associated with storms (Mürle et al., 2003; Smith et al., 2003), where the impacts of suspended fine sediment on biota are most pronounced during a sediment pulse. However, the effects of deposited sediment last long after pulse events have finished (Jones et al., 2012; 2014), potentially affect the response to further pulses. Accessibility to subsurface sediments by invertebrates strongly depends upon the hydrological and environmental characteristics of the hyporheic zone. Substrate composition strongly influences interstitial architecture, porosity, and permeability of the substrate (Maridet et al., 1992; Schmid & Schmid-Araya, 2010). These factors influence the volume of interstitial space inhabitable by invertebrates, and the spatial arrangement and size of movement pathways between particles (Stubbington, 2012). The deposition and infiltration of fine sediment (typically defined as inorganic and organic particles <2 mm in size; Wood & Armitage, 1997; Jones et al., 2012) into interstitial spaces alters bed sediment composition by reducing average particle size, filling interstices between coarser clasts and decreasing bed stability (Wood & Armitage, 1997). Smothering of the bed reduces the mobility of larger particles by restricting their protrusion (Kirchner et al., 1990) and by increasing their cohesion (Barzilia et al., 2013). The clogging of bed interstices (i.e., colmation) can restrict water and nutrient exchanges between the surface water and the hyporheic zone (Stubbington et al., 2009; Nogaro et al., 2013; Growns et al., 2016). Colmation can restrict access to the hyporheic zone for invertebrates and limits refugial space (Brunke, 1999; Descloux et al., 2013). Whilst previous studies have examined the influence of fine sediment deposition and colmation on benthic and hyporheic invertebrates (e.g., Descloux et al., 2013; Mathers et al., 2014, 2017), few studies have explored the effect of bed substrate composition on the vertical migration of invertebrates into the hyporheic zone during differing loadings of fine sediment.

Fine sediment deposition and colmation have a wide range of effects on benthic and hyporheic invertebrate community composition and trait characteristics (Jones et al., 2012; Marmonier et al., 2012; Mathers et al., 2017; Wilkes et al., 2019). Previous studies have demonstrated reductions in benthic invertebrate density, diversity, function, and modifications to the community composition caused by streambed colmation (Larsen et al., 2011; Descloux et al., 2013; Vadher et al., 2015; Mathers & Wood, 2016; Vadher et al., 2018). Invertebrate assemblages often transition from pollution-sensitive taxa dominated by Ephemeroptera, Plecoptera and Trichoptera (EPT) to taxa adapted to burrowing and tolerant of oxygen depletion, such as certain chironomids, oligochaetes, and amphipods (Hall et al., 1984; Kreutzweiser et al., 2005; Larsen et al., 2009; Larsen & Ormerod, 2010). Previous ex situ studies have examined the impacts of colmation on invertebrates accessing the hyporheic zone using sediment columns under laboratory conditions (e.g., Mathers et al., 2019). To date, no studies have explored whether invertebrates can access the hyporheic zone through a colmated bed during a fine sediment disturbance event in a field setting.

Our study explored if colmation influences invertebrate movement into the hyporheic zone during a fine sediment disturbance event. First, we examined the effects of sedimentation on benthic and hyporheic invertebrate structure by experimentally creating a coarse (i.e., a "clean") and a colmated bed. Next, we examined the influence of different doses of fine suspended sediment additions on the benthic and hyporheic community. We predicted that colmation would lower benthic and decrease hyporheic abundance and taxonomic richness and modify both benthic and hyporheic community composition before the fine sediment pulse (hypothesis H1). Secondly, we hypothesised that fine sediment loading would cause vertical migration of benthic invertebrates and, thus, increase hyporheic abundance and taxonomic richness in the coarse bed (hypothesis H2). This response would occur due to the unfavourable surface conditions. Next, we predicted that hyporheic community composition would diverge between the coarse and the colmated bed with depth and time due to the fine sediment disturbance (as access to the hyporheos as a refugium would be restricted in the colmated sediment; hypothesis H3). The results of this study will be useful in advancing understanding of how benthic invertebrates respond to varying fine sediment pressures in unpolluted and colmated streams, particularly how past sedimentation may affect the response to further fine sediment pulses. Information on the behavioural responses of invertebrates to anthropogenic sedimentation will be important for environmental managers and legislators when implementing fine sediment control strategies.

2 MATERIALS AND METHODS

2.1 Mesocosm setup

The experiment was conducted using twelve open air flow-through stream mesocosms that are fed by the Mill Stream, a side channel of the River Frome. The Frome is characterised by pool-riffle-glide morphology and possesses a meandering planform (National River Flow Archive [NRFA], 2020). The experiment comprised a 69-day colonization period followed by 2 days of manipulation (from June to August 2015). The mesocosms were positioned in four blocks comprising three steel linear stream mesocosms (0.33 m in width, 12.4 m in length and 0.30 m in depth), which were located at approximately 140° to the Mill Stream. At the start of the experiment, gravel and sand were sourced from a local quarry who obtained the sediment from the R. Frome floodplain. The coarse bed consisted of sand (<2 mm, 6.6%), gravel (10 mm, 13.3%), pebbles (20 mm, 66.7%) and cobbles (>64 mm, 13.4%) and replicated the sediment-size distribution of local chalk streams (Armitage, 1995; Ledger et al., 2008). The colmated bed comprised sand (25%), gravels (37.5%) and pebbles (37.5%). In the colmated bed, fine sediment represented 25% of the bed sediment. Each mesocosm was divided into two 6.2 m sections to provide 24 mesocosm sections. Twelve mesocosm sections were filled to a depth of 20 cm with coarse or colmated sediment (Figure 1).

Each mesocosm was fed with water from the Mill Stream by gravity via a header tank and weir. The height of the weir controlled the rate of flow into the mesocosms and was consistent across the experiment. The average current velocity in the mesocosms was 0.11 ms^{-1} ± 0.01 (mean ± SE; $n = 24$) and average water depth was 5.16 ± 0.18 cm ($n = 24$; measured once on day 99; Milner et al., 2021). The water exited the mesocosms over a small weir before flowing into a ditch, which re-entered the Mill Stream.

Invertebrates colonised the mesocosms by drift from the Mill Stream and adult oviposition (Harris, 2006). The colonisation process was aided by adding invertebrates from four 3 min kick samples collected from the Mill Stream. Benthic invertebrates were obtained from four riffles with a coarse-grained substrate that were not impacted by fine sediment deposition (Milner et al., 2021). These aliquots were added to the head of each mesocosm. Shade cloths were used to cover the mesocosms throughout the colonisation period to ensure that diatom mats did not colonise the mesocosms, which may have encouraged fine sediment settlement (Jones et al., 2014).

2.2 Sediment treatments

Sediment for the fine sediment pulse was sourced from nearby reaches in the River Frome. The sediment was frozen for 48 hours to kill any invertebrates and sieved using a 2 mm mesh to exclude larger particles. We used three sediment concentrations in the experiment: 1) no sediment (30 l of water), 2) a moderate fine sediment addition (a suspension of 15 kg sediment in 30 l of water = 0.5 kg l^{-1}), and 3) a high fine sediment addition (a suspension of 30 kg sediment in 30 l of water = 1 kg l^{-1}). We determined the moderate and high suspended fine sediment treatment would produce a concentration of 556 mg l^{-1} and 1112 mg l^{-1} (based on average velocity and depth values) across a 4-hour period. The fine sediment treatments were added at the head of each 24 mesocosm section in a crossed design with bed sediment type (Figure 1). Each 24 mesocosm section received one of three sediment concentrations (i.e., a control, a moderate and a high fine suspended sediment pulse treatment were each applied to 8 mesocosms; Figure 1). Therefore, each mesocosm was treated as an independent, experimental unit (Milner et al., 2021).

Before the fine sediment disturbance, a five-litre sediment sample was taken randomly from the coarse and colmated bed to ensure consistency within the bed sediment types between the mesocosm sections, and to identify differences in particle size between the coarse and the colmated sediment. The sediment was oven dried at 60 °C and sieved into the particle size classes: <0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 31.5, 45 and 63 mm or greater. Each size fraction was weighed to identify the range of particle sizes within each bed sample.

2.3 Benthic and hyporheic invertebrate sampling

Two sampling tubes were inserted into the bed sediments of each mesocosm section at an upstream and downstream location (please see Mathers & Wood, 2016 for a conceptualisation of the sampling tubes). The sampling tubes were inserted to depths of 5 and 18 cm and were *c*. 20 cm apart. The sampling tubes were made from PVC piping and had a diameter of 12 mm. Each sampling tube had four 5 mm holes on the vertical side, which were located 10 mm from the bottom. The top and the bottom of the tubes were sealed using a foam bung between sampling occasions.

Hyporheic invertebrates were collected from the sampling tubes on the day prior, during (i.e., within 4 hours of the start of the fine sediment disturbance event), and directly following fine sediment addition (i.e., 24 hours post the fine sediment disturbance event); hereafter, referred to as before, during, and after. Prior to sampling, the foam bung was removed from the base of the sampling tube. This method drew water from the zone immediately adjacent to the four 5 mm holes (i.e., at either 5 or 18 cm). Hyporheic invertebrates were sampled by collecting 500 mL of water from the PVC tube. After collection, water and invertebrates were sieved through a 250 µm mesh and the remaining sample preserved in 99% industrial methylated spirits (IMS).

Benthic invertebrates were collected using a surber sampler (0.2 m^2, 250 µm mesh net) at an upstream and a downstream location in each mesocosm section before and after fine sediment loading. Bed sediments were disturbed using a metal rod over a 2-min time period that allowed invertebrates to flow into the downstream net. In total, 288 hyporheic samples (i.e., 2 different depths x 2 locations within the mesocosm section x 24 mesocosm sections x 3 sampling occasions) and 96 benthic samples were collected (i.e., 2 locations within each mesocosm section x 24 mesocosms x 2 sampling occasions). All invertebrate samples were preserved in 99% IMS and returned to the laboratory for processing and identification. Most invertebrates were identified to species, although, Oligochaeta and Hydrachnidia were identified at the class level.

2.4 Data analysis

At the beginning of the experiment, differences in bed particle size between the colmated and the coarse bed were tested using a one-way Analysis of Similarity (ANOSIM). Bed particle size data was square root transformed, normalised and a Euclidean distance measure was used to minimise any influence of skewed distributions (Clarke & Gorley, 2006). A total of 9999 randomisations were used to test for significant relationships between bed particle size and the proportion of fine particles. There was a significant difference in the proportion of particle sizes (ANOSIM; $r = 0.907$, $p < 0.001$) between the coarse and the colmated bed at the beginning of the experiment: the findings are described in detail by Milner et al., (2021).

Benthic and hyporheic abundance and taxonomic richness were determined for all samples. Invertebrate abundance data was log10 (x+1) transformed to reduce skewed distributions and normalise residuals. Abundance and taxonomic richness from all benthic and hyporheic (at 5 and 18 cm) were analysed separately due to differences in sampling techniques. First, a repeated-measures analysis of variance (RM-ANOVA) was used to identify any significant effects of bed sediment (i.e., the coarse and the colmated bed) on invertebrate abundance and taxonomic richness in (i) the benthic, and (ii) the hyporheic zone before fine sediment loading.

The RM-ANOVA incorporated block (1-4) and mesocosm section (1-24; to account for any potential positional effect caused by the mesocosms) as within subjects factors, and bed sediment and depth (i.e., 5 and 18 cm for the hyporheic analysis) as between-subjects factors. This analysis aimed to examine the impacts of prior colmation on benthic and hyporheic abundances and taxonomic richness before the fine sediment pulse.

Further RM-ANOVAs examined any differences in (i) benthic and (ii) hyporheic abundances and taxonomic richness due to the fine sediment disturbance event. Sediment treatment (i.e., the control, moderate and high sediment additions), depth, time (i.e., before, during and after), and bed sediment were included as between-subjects factors, and block and mesocosm section were incorporated as within-subjects factors. A general linear model (GLM) highlighted any interactive effects on abundance and taxonomic richness caused by a combination of these main factors. The GLM procedure in the SAS 9.4 statistics package was used for the analysis.

We used a permutational multivariate analysis of variance (PERMANOVA) to test any differences in the benthic and hyporheic invertebrate community composition between bed sediment type before the fine sediment disturbance. The main factors of interests were bed sediments and depth (i.e., 5 cm and 18 cm for the hyporheic analysis), which were included as fixed factors. Block and mesocosm section were treated as random factors and were included as random factors in all subsequent PERMANOVA models. Next, we investigated any differences in the benthic and hyporheic community composition caused by fine sediment loading. These PERMANOVA models incorporated time (before, during and after), fine sediment treatment (i.e., the control, moderate and high sediment additions), depth (for the hyporheic analysis), and bed sediments as fixed factors. Prior to all analyses, invertebrate abundances were log transformed to decrease the influence of the most abundant taxa, and Bray-Curtis distance between samples was used to create similarity matrices for the invertebrate data. In addition, the abundances of common taxa (defined as individuals accounting for >1% of the community composition in all samples) were used for the analyses. Planned contrasts were used to allow examination of main factors (i.e., depth, time, fine sediment treatment and bed sediment). PERMANOVA was performed using 9999 permutations and tested for differences in benthic and hyporheic invertebrate community composition caused by the main factors and their interaction. A non-metric multidimensional scaling (NMDS) ordination using 50 randomised starts was used to visually display the PERMANOVA results (Clarke, 1993). Similarity Percentage (SIMPER; Clarke, 1993) routine was carried out to identify any taxa causing differences between the key treatments identified by PERMANOVA. The multivariate analyses were performed using PRIMER v.7 and the PERMANOVA+ add-on (Anderson et al., 2008).

3 RESULTS

3.1 Effects of prior colmation on benthic and hyporheic communities

Before fine sediment addition, benthic abundance and richness were marginally significantly higher in the coarse than the colmated sediments (Table 1). The abundance of hyporheic invertebrates was higher in the colmated sediment, but again the difference was only marginally significant. Depth had a substantial effect on hyporheic abundance and hyporheic taxonomic richness, which were significantly higher at 5 than at 18 cm (ANOVA; $p < 0.05$).

Before fine sediment addition, benthic invertebrate community composition was similar between the coarse and the colmated bed (PERMANOVA; F = 2.29, $p > 0.05$). Hyporheic community composition varied significantly with depth (PERMANOVA; F = 3.44, $p < 0.05$), but not bed sediment (PERMANOVA; F = 1.1, $p > 0.05$). This output was supported visually by a NMDS ordination (Figure 2). Most upper surface samples were positioned in the top part of the ordination, with the lower subsurface samples located towards the bottom of the NMDS plot, but their distributions overlapped with scatter present (Figure 2).

The top five invertebrate taxa comprising the benthic community composition before fine sediment addition were Tanypodinae (accounting for 29.7%), Tanytarsini (22.44%), Oligochaeta (13.7%), *A. aquaticus* (7.6%) and *H. pellucidula* (5.7%). These five taxa accounted for 79.1% of the benthic community composition. The top five invertebrate taxa (identified by SIMPER) driving significant differences in hyporheic community composition between the two sedimentary layers were Cyclopoida (contributing 17.96% of the dissimilarity), Tanypodinae (15.84%), *C. pseudogracilis* (13.82%), *A. aquaticus* (11.97%) and Oligochaeta (10.3%). Cyclopoida and Tanypodinae were more abundant at 5 cm, whilst *C. pseudogracilis*, *A. aquaticus* and Oligochaeta occurred in greater numbers at 18 cm.

3.2 Response to the fine sediment pulse: Vertical migration of invertebrates

Benthic abundance (F = 7.69, $p < 0.001$) and taxonomic richness (F = 9.22, $p < 0.001$) were significantly higher after the fine sediment pulse. Abundances of Tanypodinae, Tanytarsini, *A. aquaticus*, *G. pulex* and baetids doubled after the fine sediment loading. Tanypodinae and Tanytarsini dominated the benthic community composition before and after the disturbance (55.1% and 58.6% respectively). Time (denoting the effect of the fine sediment pulse) and block significantly affected benthic abundances (F value = 4.91, $p < 0.001$), but bed sediments and fine sediment treatment had no influence on benthic abundances or taxonomic richness (all p values >0.5). There were no other significant two or three-way interactions for benthic abundance or taxonomic richness.

Time, fine sediment treatment and bed sediments had no significant effect on the abundance and richness of hyporheic invertebrates (Table 2; Figure 3). However, hyporheic abundance and taxonomic richness significantly varied with depth after the fine sediment pulse (Table 2). The two-way interactions involving time and the other main factors (i.e., fine sediment treatment, bed sediments and depth) all significantly influenced hyporheic abundance (Table 2). Hyporheic abundance was significantly higher at 5 than 18 cm at the start of the experiment (Figure 3a). During the fine sediment pulse, hyporheic abundance declined at 5 cm but rose at 18 cm, which resulted in similar hyporheic abundances in both sediment layers (Figure 3a). Hyporheic abundances increased at 5 cm after the fine sediment pulse and were significantly higher than hyporheic abundances at 18 cm (Figure 3a). RM-ANOVA also revealed the two-way interaction of time and fine sediment treatment significantly influenced hyporheic abundances. During the fine sediment pulse, hyporheic abundance was significantly lowest in the control and highest in the high fine sediment treatment but did not vary before or after fine sediment loading (Figure 3c).

Depth, block, and the two-way interactions of time x fine sediment treatment, and time x depth significantly affected hyporheic taxonomic richness (Table 2). Before the fine sediment pulse, hyporheic taxonomic richness was significantly higher at 5 than 18 cm (Figure 3b). At 5 cm, hyporheic taxonomic richness decreased during but increased 24 hours after the fine sediment pulse. In the lower sedimentary layer, taxonomic richness increased during and remained higher after the fine sediment pulse compared to pre sediment input values. Fine sediment treatment (Figure 3d) and bed sediments (Figure 3f) did not influence hyporheic taxonomic richness, and none of the three or four-way interactions for both hyporheic abundance and taxonomic richness were significant, although replication and hence, statistical power was lower at these higher levels.

3.3 Temporal patterns in benthic and hyporheic community composition with fine sediment loading

Benthic invertebrate community composition varied with time (PERMANOVA; $F = 4.6$, $p < 0.05$), indicating change over the duration of the experiment. Bed sediments ($F = 1.63$, $p > 0.05$) and fine sediment treatment ($F = 1.85$, $p > 0.05$) were not influential on benthic invertebrate community composition. None of the two or three-way interactions between time, bed sediments, fine sediment treatment and block were significant (all p values >0.05) for the benthic community structure. However, time, bed sediments, depth and block strongly influenced hyporheic community composition (Table 3). The two-way interaction of time and depth was significant, as was the interaction of time and block, which was of less interest to the experimental design (Table 3). Planned contrasts indicated that the invertebrate hyporheic community composition differed before and during, and before and after the fine sediment pulse. The two-way interaction of time and depth revealed significant changes in hyporheic community composition i) before and during the fine sediment pulse with depth, ii) before and after with depth, iii) during and after with depth, and iv) between sampling occasions and depth profiles (Table 3). The influence of time, bed sediments and depth on hyporheic community composition was supported visually by the NDMS ordination (Figure 4). The NMDS ordination indicated that the degree of separation between invertebrate groups varied, and although no group had a distinct distribution, divergences were significant (Table 3).

A SIMPER analysis showed that the top 5 taxa accounting for differences in hyporheic community composition at 5 cm before and during the fine sediment pulse were *C. pseudogracilis* (17.32%), Cyclopoida (15.88%), Tanypodinae (14.09%), *A. aquaticus* (11.02%) and Tanytarsini (9.62%). Cyclopoida, Tanypodinae and Tanytarsini were more abundant before than during the fine sediment pulse. Similar patterns occurred in hyporheic community composition before and after the fine sediment pulse. Cyclopoida (16.51%), Tanypodinae (12.81%), Tanytarsini (10.9%) were present in higher numbers before the fine sediment pulse, whereas *C. pseudogracilis* (13.25%) and Daphniidae (10.26%) occurred in higher numbers after the fine sediment pulse.

At 18 cm, SIMPER revealed that *C. pseudogracilis* (30.07%), Cyclopoida (23.93%), *A. aquaticus* (14.04%), Tanypodinae (11.39%) and Oligochaeta (11.11%) drove variation in hyporheic community composition before and during the fine sediment pulse. *A. aquaticus* and Oligochaeta were more abundant before the fine sediment pulse, whilst C. *pseudogracilis*, Cyclopoida and Oligochaeta dominated the community composition after the fine sediment pulse. Examination of the hyporheic community composition at 18 cm before and after the fine sediment pulse revealed *C. pseudogracilis* (27.95%), Cyclopoida (26.73%), *A. aquaticus*

(14.3%), Oligochaeta (10.98%) and Ostracoda (10.8%) were responsible for significant differences in invertebrate structure. *A. aquaticus* and Oligochaeta occurred in higher numbers before the fine sediment pulse compared with *C. pseudogracilis*, Cyclopoida and Ostracoda.

4 DISCUSSION

4.1 Impacts of colmation on invertebrate communities

Fine sediment deposition and infiltration of particles into subsurface sediments influences habitat availability, heterogeneity, and the spatial distribution of benthic and hyporheic invertebrates (Weigelhofer & Waringer, 2003; Mermillod-Blondin et al., 2015). At the start of the experiment, we expected the coarse bed to support higher benthic and hyporheic abundances and taxonomic richness compared with the colmated bed. Coarse sediments generally possess increased interstitial space, more habitat and higher permeabilities that support greater oxygen, organic matter, and nutrient exchanges (Dole-Olivier et al., 1997; Strayer et al., 1997; Stubbington, 2012; Jones et al., 2015). Despite a significant difference in the proportion of bed particle sizes between the coarse and the colmated bed (6.6% *cf* 25% fines), we found limited evidence to support our first hypothesis that colmation influences benthic and hyporheic abundance and taxonomic richness: differences were small and of marginal significance (ANOVA; $p = 0.079$ for benthic abundance; $p = 0.055$ for benthic taxonomic richness; $p = 0.057$ for hyporheic abundance). Furthermore, we observed no differences in benthic invertebrate community composition between the coarse and the colmated bed. Previous studies exploring the effects of colmation on abundance, taxonomic richness and community composition have reported inconsistent findings with some studies finding lower benthic and hyporheic abundance, taxonomic richness and diversity with increasing colmation (Descloux et al., 2013; Vadher et al., 2015; Vadher et al., 2017; Mathers et al., 2019), whereas others have observed no effect (Kaller & Hartman, 2004; Downes et al., 2006), and some experiments have found increased abundances (Matthaei, et al., 2006). The differing outputs of these studies may be due to variation in the proportion of fine particles within the bed sediments, their composition, and/or the flow of water all that may influence the suitability of the hyporheic zone for invertebrates.

4.2 Impact of the fine sediment pulse on benthic invertebrates

Benthic abundance and taxonomic richness increased after the fine sediment pulse. Higher abundances were largely due to an increase of a factor 2 of Tanypodinae and Tanytarsini individuals. The two taxa accounted for over half of the benthic community before and after the pulse (55.1% and 58.6% of the community composition), and both taxa are tolerant of fine sediment pollution (Murphy et al., 2015; Turley et al., 2015). Tanypodinae and Tanytarsini were present in the hyporheic zone before the fine sediment pulse. Abundances of both taxa decreased during and increased after the fine sediment pulse, indicating a two-way interaction with the benthic and the hyporheic zone. Previous studies have revealed that rivers with colmated sediments are often characterised by chironomids and Oligochaeta (Lenat et al., 1979, 1981; Richards et al., 1993; Angradi, 1999; Larsen & Ormerod, 2010; Jones et al., 2012), including Tanytarsini (Descloux et al., 2013).

In the benthic layer, Baetidae was also found to be more abundant after the fine sediment disturbance. Baetidae have been identified as tolerating up to 70% of fine particles within a substrate (Lemly, 1982). Many studies have highlighted that the genus *Baetis* spp. appear even more tolerant to colmation. Descloux et al., (2013) found increased Baetidae densities in both benthic and hyporheic sediments with colmation. In our study, the increase in Tanypodinae, Tanytarsini and Baetidae may help to explain the observed increased abundance in the benthic zone with fine sediment loading.

Before the fine sediment pulse, we observed a pronounced gradient in the hyporheic community with depth, in terms of community composition, abundance and richness. Abundance and richness were significantly higher at 5 cm than 18 cm. Cyclopoida and Tanypodinae dominated the hyporheic community at 5 cm, whereas *C. pseudogracilis*, *A. aquaticus* and Oligochaeta occurred in greater numbers at 18 cm. Cyclopoida are small crustaceans of approximately 3 mm in size and are capable of rapid movement through sedimentary layers (Dobson et al., 2012). Their small size and dispersal abilities help explain their dominance in the upper sediments of the hyporheic zone and ability to escape surface disturbances. *A. aquaticus* and Oligochaeta disperse by crawling and burrowing respectively. Oligochaetes are active bioturbators and create burrow networks within sediment matrices (van Regteren et al., 2017). The locomotion traits of *A. aquaticus* (i.e., a crawler) and Oligochaeta (a burrower) allow movement through subsurface sediments to avoid unfavourable events.

4.3 Vertical migration of invertebrates in response to fine sediment loading

In the hyporheic zone, invertebrate abundance typically declines with depth (Coleman & Hynes, 1970; Williams & Hynes, 1974) due to decreases in habitat availability and homogenisation of interstitial habitats (Descloux et al., 2013). Most invertebrates are mobile and can move bidirectionally between benthic and hyporheic sediments but require a minimum pore space for vertical movement (Descloux et al., 2013). Past work has found equivocal evidence for the hyporheic refuge hypothesis during surface disturbances (Dole-Olivier et al., 1997; Stubbington et al., 2011; Vadher et al., 2015). These ambiguous findings have been linked to differences in environmental characteristics, especially bed sediment composition (Stubbington, 2012). Fine sediment deposition and colmation can restrict vertical migrations of invertebrates between the benthic and the hyporheic zone by modifying habitat characteristics, such as by decreasing average particle size, filling interstices between larger particles, reducing water flow velocity and lowering inputs of resources from the surface (Wood & Armitage, 2007; Descloux et al., 2013). Seal formation due to colmation may even prevent invertebrates accessing the hyporheic zone and refugial habitat (Brunke, 1999). Thus, colmation can reduce the hyporheic zone functioning as an ecological refuge during instream disturbances (Palmer et al, 1992; Marmonier et al., 1993; Brunke & Gonser, 1997; Dole-Olivier et al., 1997).

We found support for our second hypothesis that a fine sediment pulse would cause vertical migration of benthic invertebrates and an increase in the abundance and taxonomic richness of hyporheic invertebrates. During the fine sediment pulse, hyporheic abundance and richness increased significantly with fine sediment loading (lowest in the control and highest in the high fine sediment loading). This effect was not apparent before and after the fine sediment pulse. Our study also revealed a change in the depth distribution of hyporheic invertebrates during the fine sediment pulse. At the start of the experiment, abundance and richness of hyporheic invertebrates were higher at 5 than 18 cm but were indistinguishable between the two depths during the fine sediment disturbance. This finding indicates vertical movement of invertebrates through the hyporheic zone: abundance and richness decreased at 5 cm and increased at 18 cm. After the fine pulse of sediment, hyporheic abundance and richness were again higher at 5 than 18 cm, indicating a return to the pre-disturbance depth distribution. These two results combined clearly indicate that the invertebrates were using the hyporheic zone as a refuge as a behavioural response to the fine sediment pulse.

Our data also provides support for our third hypotheses that a fine sediment disturbance would cause divergence of the hyporheic community composition. Significant changes in hyporheic community composition occurred in both sedimentary layers before and during, before and after, and during and after the fine sediment pulse, and with depth. In the upper sedimentary layer, Cyclopoida, Tanypodinae and Tanytarsini occurred in higher numbers before, whereas *C. pseudogracilis* and Daphniidae increased after the fine sediment pulse. At 18 cm, *A. aquaticus* and Oligochaeta dominated the hyporheic community composition before the fine sediment pulse, whilst *C. pseudogracilis,* Cyclopoida and Ostracoda increased after. These findings illustrate that invertebrates moved vertically into subsurface sediments during the fine sediment pulse and support the hyporheic zone acting as an ecological refuge.

4.4 Influence of fine sediment treatment and bed sediment on vertical migration

Here we have shown that invertebrates used the hyporheos as a refuge during a fine sediment pulse. The increase in the abundance of hyporheic invertebrates to fine sediment loading and changes in the vertical distribution during the fine sediment disturbance event indicates vertical movement of individuals and the accessibility of movement pathways between particles. Previous ex situ studies using experimental sediment tanks and columns have found that higher fine sediment loadings restrict invertebrates from accessing the deeper subsurface sediments (Vadher et al., 2015; Vadher et al., 2018; Mathers et al., 2019). Generally, pore spaces fill steadily from the base of a sediment column via unimpeded percolation (Diplas & Parker, 1992), and thus, restrict invertebrate movement to the deeper parts of the substrate (Mathers et al., 2019). Whilst there may have been some sorting of the vertical distribution of particles during the experiment such that finer particles accumulated at depth (see Jones et al. 2015), we manipulated the particle size distribution of the bed (6.6% *cf.* 25% fines) and expected bed substrate composition to influence the response of invertebrates to the sediment pulse through reduced access to the deeper sections of the mesocosms due to clogging of interstitial spaces. The lack of a strong effect of bed sediment and an interaction between bed sediment, depth, and time on hyporheic invertebrate abundance and richness suggests that this did not occur.

At the start of the experiment, there was a marginal effect of bed sediments on the abundance of benthic and hyporheic invertebrates and a clear gradation of hyporheic invertebrates with depth. However, there was no interaction between bed sediments and depth indicating that the depth distribution of hyporheic invertebrates was similar between the coarse and the colmated bed. As the experiment involved addition of more fine sediment in a pulse, we may have expected this fine sediment to lead to further clogging of interstices; if this occurred, it was insufficient to restrict access for invertebrates as the interactions between bed sediments, depth and time, and between bed sediment, depth, fine sediment treatment and time were not significant. We conclude that differences in substrate particle size between our treatments were insufficient to impact the permeability of the substrate for hyporheic invertebrates, despite the colmated bed comprising 25% sand (*cf.* 6.6 % in the coarse substrate treatment).

Bed sediment composition and porosity effect hydrological exchanges and invertebrate movement, with colmation decreasing the strength of hydrological and fauna exchanges whilst coarse substrates permit free water and invertebrate movement (Brunke, 1999; Hancock, 2002). Clogging of interstitial pathways also decreases the connectivity between the benthic and the hyporheic zone, and generally restricts invertebrate vertical movements (Vadher et al., 2015). In our study, we speculate that movement pathways between particles in the colmated bed were still accessible for invertebrates. This finding contrasts with previous ex situ studies that have found invertebrate movement into subsurface sediments decreased or stopped after fine sediment volumes exceeded a specific percentage (e.g., Vadher et al., 2015; 2018).

4.5 Future research needs and management applications

In contrast with previous ex situ studies (e.g., Vadher et al., 2015, 2018; Mathers et al., 2019), our research did not identify a strong influence of our experimental colmation on benthic or hyporheic community dynamics. At the start of our experiment, the coarse and colmated bed sediments possessed distinct particle size distributions with clear differences in the percentage of fine sediment. However, the proportion of fine sediment in both bed sediments is likely to have increased over time as fine sediment was deposited in the mesocosms from the inflowing water (Jones et al., 2015). Further research is needed to examine the recovery of physical habitat heterogeneity, and benthic and hyporheic invertebrates after fine sediment inputs. Flow is a key factor influencing the colmation of sediments and their recovery, but the relationship between flow and colmation is complex (Jones et al., 2015). Peak flows typically deliver large amounts of fine sediment, while frequently occurring floods normally winnow out fine sediments (Mürle et al., 2003; Smith et al., 2003). Future research is required to identify the flow threshold needed to remove fine sediment and identify whether the impacts of fine sediment on benthic and hyporheic habitats and invertebrate communities are short- or long-lived. Furthermore, it would be valuable to find out whether the fine sediment on the bed remains in situ or is removed gradually by fluctuating flows. This information would be invaluable for river hyporheic restoration and effective catchment management strategies (Mathers et al., 2017).

Our study provided conclusive evidence of a behavioural response by invertebrates (albeit indirect evidence through rapid changes in their distribution) where they use subsurface sediments as a refuge during adverse surface conditions. The hyporheic zone and other refuge habitats have an important ecological role in the persistence and resilience of fauna to instream disturbances. The availability, accessibility and ecological integrity of refuges is of growing importance with ongoing climate change, increased hydrological extremes and anthropogenic sedimentation (Jones et al., 2012; Extence et al., 2013). Our study indicated vertical migration of benthic invertebrates into subsurface sediments despite colmation, although our experiment was carried out in a single season and vertical invertebrate movements are highly changeable with season (with larvae growth; Puig et al., 1990) and hydrological events (Dole-Olivier et al., 1997). We advocate management actions aimed at improving streambed porosity to enhance the availability of refuges for aquatic biota. Management actions should address entry of fine sediment into river ecosystems by creating sediment detention ponds/basins and planting riparian vegetation to stabilise riverbanks (Verstraeten & Poesen, 2000). In-stream measures could also include replenishing depleted coarser sediments (McManamay et al., 2010) and installing flow deflectors to increase hydraulic efficiency to transport fine sediments (Michel et al., 2014).

5 CONCLUSIONS

Our study highlights the ecological role of the hyporheic zone as a refuge for benthic invertebrates during a fine sediment disturbance. In contrast with previous work, we found benthic invertebrates could access deeper subsurface sediments within the hyporheic zone in coarse and colmated sediments. We also observed increased use of the hyporheos as a refuge by benthic invertebrates with increasing load of suspended fine sediment. We conclude that movement pathways between particles were accessible and allowed bidirectional vertical migrations of individuals between the benthic and the hyporheic zone. Refuges, including subsurface sediments help promote the survival and persistence of invertebrate communities during adverse conditions in the surface stream. Access to and within the hyporheic zone supports community resilience to disturbance events. Management strategies sustaining and enhancing streambed porosity will be increasingly important with ongoing climate change and increased anthropogenic sedimentation.

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TABLE 1 Effects of bed substrate on benthic and hyporheic invertebrate abundance and taxonomic richness before the fine sediment disturbance event. Significant results from ANOVA are shown in bold ($p < 0.05$); marginally significant results ($p < 0.1$) are shown in italics.

		Benthos				**Hyporheos**			
		Abundance		**Richness**		**Abundance**		**Richness**	
Source of variance	**df**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Block (Bl)	3	2.03	0.124	1.98	0.132	3.81	**0.013**	2.37	*0.076*
Bed sediments (BS)	1	3.24	*0.079*	3.91	*0.055*	3.72	*0.057*	1.39	0.808
Upstream/downstream	4	0.12	0.891	0.2	0.817	1.34	0.262	1.25	0.296
Depth (D)	1					19.44	**<0.001**	20.92	**<0.001**
BS x D	1					1.18	0.281	0.92	0.341

TABLE 2 Effects of the fine sediment pulse (time), fine sediment treatment and bed sediments on hyporheic invertebrate abundance and taxonomic richness. Significant results from RM-ANOVA are shown in bold ($p < 0.05$); marginally significant results ($p < 0.1$) are shown in italics.

		Hyporheic abundance		**Hyporheic taxonomic richness**	
Source of variance	**df**	**F value**	**p value**	**F value**	**p value**
Time (T)	2	2.16	0.119	0.27	0.767
Fine sediment treatment (FST)	2	0.42	0.656	0.38	0.685
Bed sediments (BS)	1	0.19	0.668	0.06	0.808
Depth (D)	1	14.58	**<0.001**	18.07	**<0.001**
Block (Bl)	3	5.36	**0.002**	3.3	**0.025**
T x Bl	6	2.17	**0.049**	1.76	0.113
T x FST	4	3.17	**0.016**	3.41	**0.011**
T x BS	2	3.13	**0.047**	1.28	0.282
T x D	2	5.14	**0.007**	4.37	**0.014**
FST x BS	2	2.95	*0.059*	2.13	0.126
FST x D	2	0.65	0.525	0.29	0.751
BS x D	1	1.64	0.205	1.44	0.235
T x FST x BS	4	0.26	0.904	0.37	0.831
T x FST x D	4	0.71	0.586	0.69	0.602
T x BS x D	2	0.02	0.983	0.06	0.941
FST x BS x D	2	0.98	0.38	1.56	0.218
T x FST x BS x D	4	1.34	0.258	0.94	0.442

TABLE 3 Influence of the fine sediment pulse on hyporheic community composition. Significant differences are shown in bold ($p < 0.05$); marginally significant results ($p < 0.1$) are shown in italics.

		Community composition	
Source of variance	**df**	**F value**	**p value**
Bed sediments (BS)	1	2.86	**0.017**
Time (T)	2	3.2	**<0.001**
Depth (D)	1	11.4	**<0.001**
Block (Bl)	3	6.1	**<0.001**
T x D	2	3.9	**<0.001**
T x BS	2	3.85	0.678
T x Bl	6	2.22	**<0.001**
D x BS	1	0.69	0.63
D x Bl	3	1.64	*0.06*
BS x Bl	3	0.79	0.68
T x D x BS	2	0.59	0.81
T x D x Bl	6	1.04	0.42
T x BS x Bl	6	0.84	0.70
D x BS x Bl	3	1.58	*0.08*
T x D X BS x Bl	6	0.88	0.64
Planned contrasts			
Time			
Before x During	1	3.12	**0.011**
Before x After	1	4.23	**<0.001**
During x After	1	2.07	*0.074*
Time x Depth			
Before x During x D	1	3.22	**0.009**
Before x After x D	1	5.78	**<0.001**
During x After x D	1	2.35	**0.05**
Time x 5 cm x 18 cm	2	3.86	**<0.001**

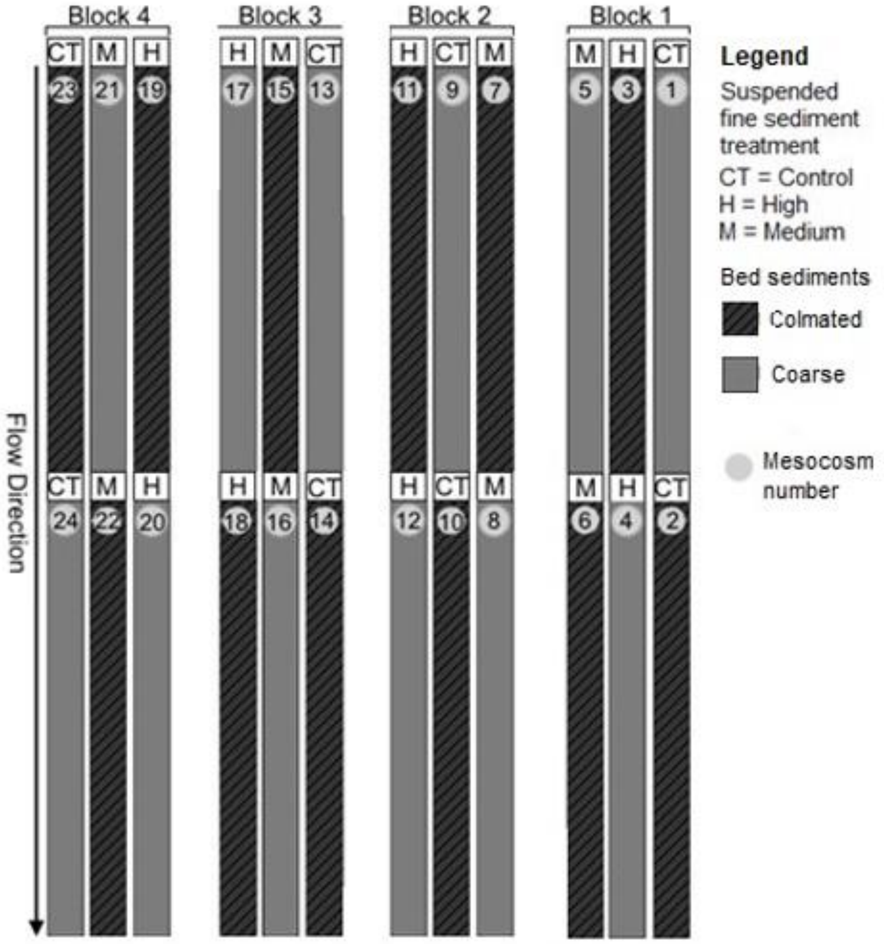

FIGURE 1 Arrangement of bed sediment types and fine sediment treatments in the stream mesocosms (Milner et al., 2021).

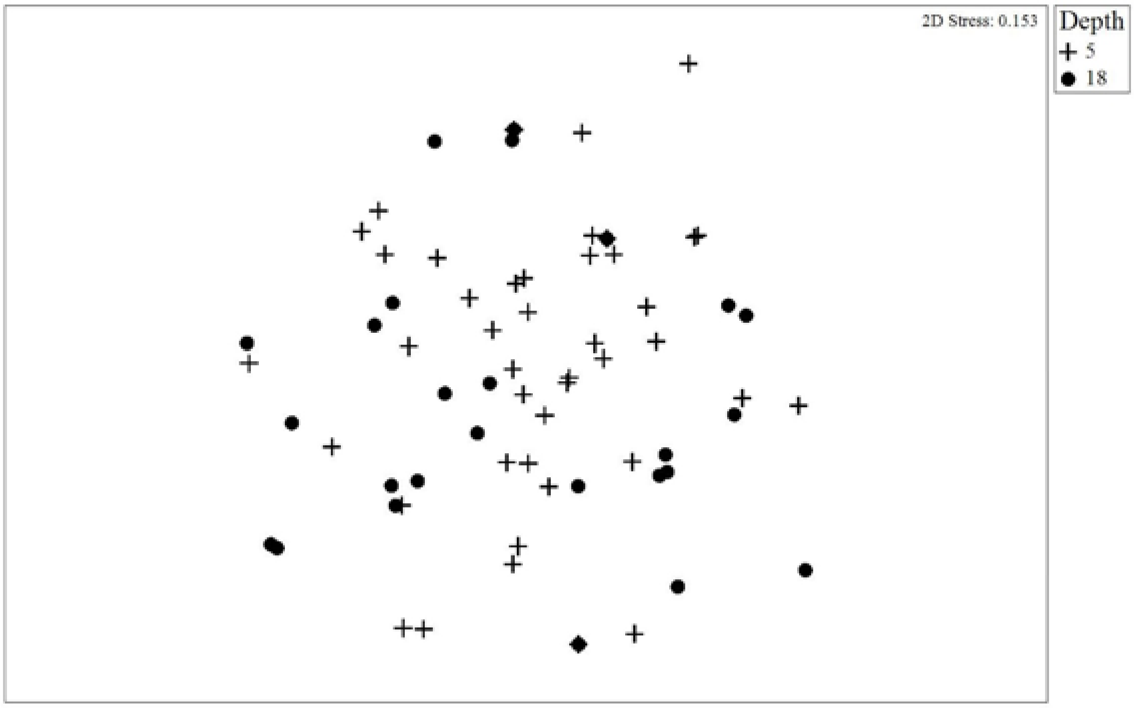

FIGURE 2 NMDS ordination plot of hyporheic invertebrate community composition prior to the fine sediment pulse.

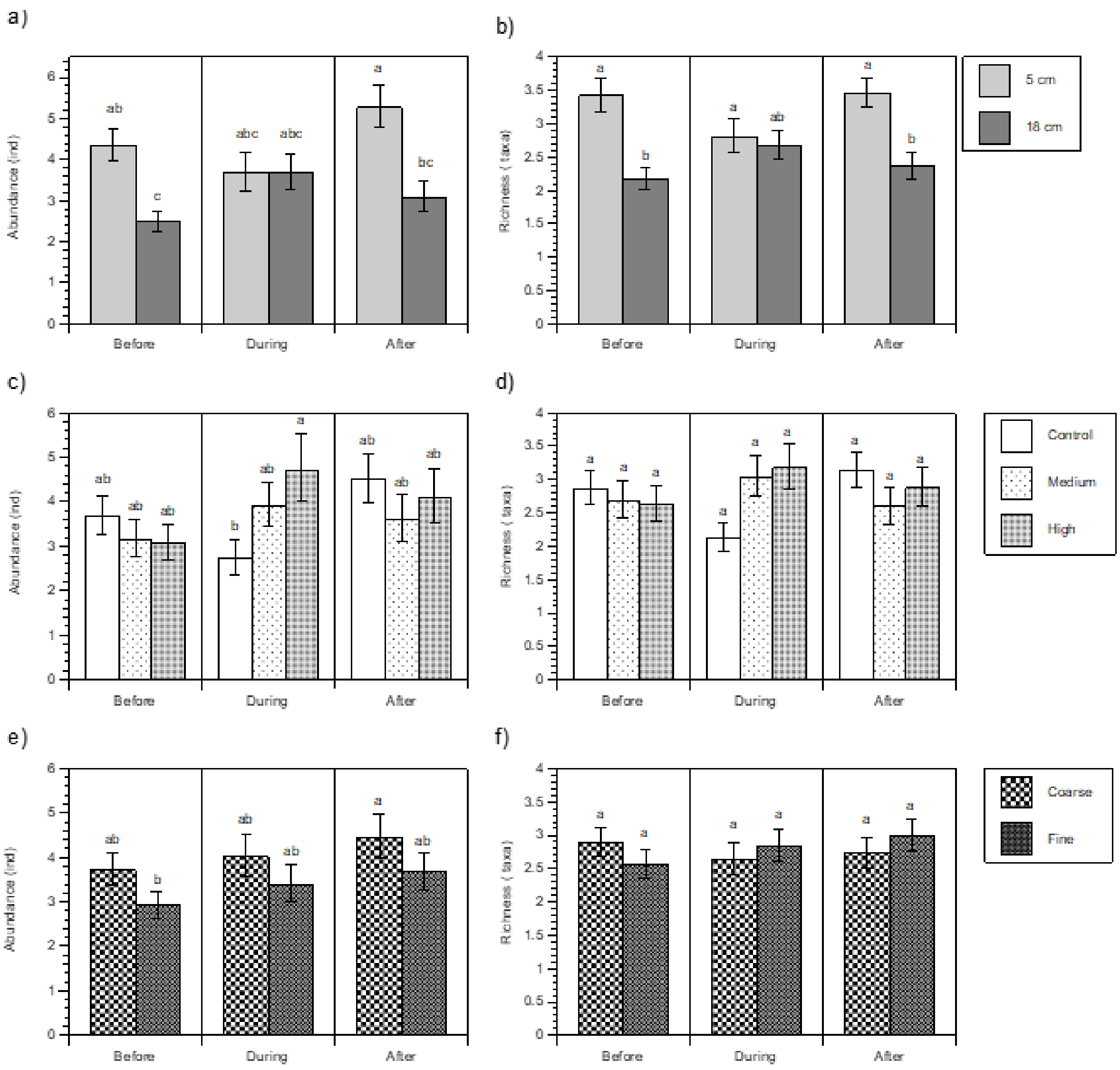

FIGURE 3 Variation in abundance and richness of hyporheic invertebrates over the experiment in response to depth (a & b), fine sediment treatment (c & d) and bed sediments (e & f). Results of Tukey's post hoc tests are shown, where mean values sharing the same letter are not significantly different. For statistical results see Table 2. Note, bed sediments had no significant effect on hyporheic richness (f).

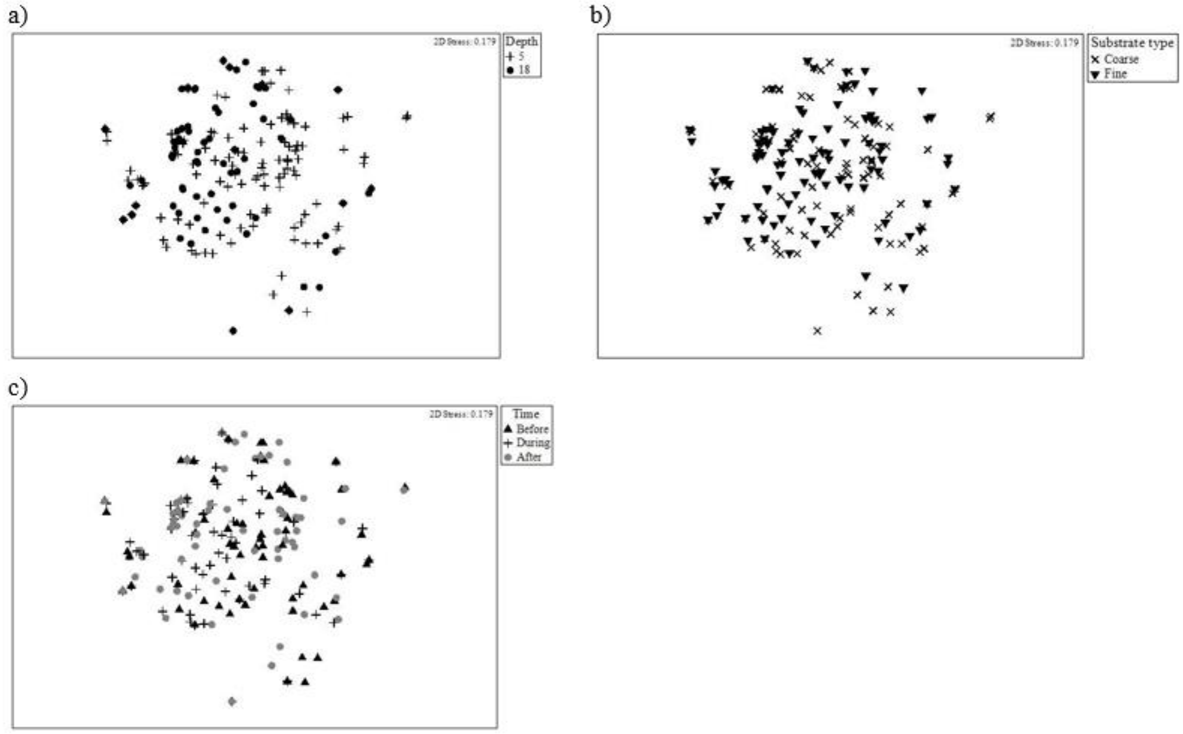

FIGURE 4 NMDS ordination plots of hyporheic invertebrate community composition by a) depth (i.e., at 5 and 18cm, b) bed sediment and c) time.