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RESEARCH ARTICLE

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Micro-computed tomography scanning approaches to quantify, parameterize and visualize bioturbation activity in clogged streambeds: A proof of concept

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Abstract

Fine particle clogging and faunal bioturbation are two key processes co-occurring in the hyporheic zone that potentially affect hyporheic exchange through modifications in the sediment structure of streambeds. Clogging results from excessive fine sediment infiltration and deposition in rivers, and it is known to decrease matrix porosity and potentially reduce permeability. Faunal bioturbation activity may compensate for the negative effect of clogging by reworking the sediment, increasing porosity, and preventing further infiltration of fines. Although both processes of clogging and bioturbation have received significant attention in the literature separately, their combined effects on streambed sediment structure are not well understood, mostly due to the lack of a standard methodology for their assessment. Here, we illustrate a novel methodology using X-ray computed tomography (CT), as proof of concept, to investigate how, together, clogging and bioturbation affect streambed porosity in a controlled flow-through flume. By visualising gallery formations of an upward conveyor macroinvertebrate; *Lumbriculus variegatus* as a model species, we quantified bioturbation activity in a clogged streambed, focusing on orientation, depth, and volume at downwelling and upwelling areas of the flume. Gallery creation increased the porosity of the streambed sediment, suggesting a potential improvement in permeability and a possible offset of clogging effects. We illustrate the promising use of X-ray CT as a tool to assess bioturbation in clogged streambeds, and the potential role of bioturbation activity supporting hyporheic exchange processes in streambeds, warranting further studies to understand the extent of bioturbation impacts in natural systems.

KEYWORDS
bioturbation, clogging, hyporheic zone, micro-CT scanning, river ecosystem function, streambed sediment porosity, water–sediment interface, X-ray tomography

1 | INTRODUCTION

The hyporheic zone (HZ) is the saturated region lying below and adjacent to the river streambed, where groundwater may intermix with surface water enabling the vertical and horizontal exchange of water, nutrient, sediment, and other waterborne material through upwelling and downwelling flow (Boulton, Findlay, Marmonier, Stanley, & Valett, 1998; Brunke & Gonser, 1999; Datry & Larned, 2008; Findlay, Strayer, Goumbala, & Gould, 1993; Malard, Ferreira, Dolédec, & Ward, 2003). Hyporheic exchange facilitates complex, dynamic, and simultaneously occurring hydraulic, thermal, biogeochemical, and ecological processes critical for overall freshwater system function (Arrigoni et al., 2008; Boano, Harvey, & Marion, 2014; Fischer, Kloep, Wilzcek, & Pusch, 2005; Lewandowski et al., 2019; Ward, 2016; Zarnetske, Haggerty, Wondzell, & Baker, 2011). Streambed sediment structure provides the physical framework where hyporheic exchange occurs, with streambed permeability being a dominant hydraulic driver of these processes (Bardini, Boano, Cardenas, Revelli, & Ridolfi, 2012; Burkholder, Grant, Haggerty, Khangaonkar, & Wampler, 2008; Mauclaire, Schürmann, & Mermillod-Blondin, 2006). Permeability is the ability of a porous media to transmit fluid (Shepherd, 1989). It varies over several orders of magnitude temporally and spatially (Buss et al., 2009; Stewardson, Grant, & Marusic, 2011; Wörman, Packman, Johansson, & Jonsson, 2002) and depends largely on the sediment properties. Both sediment structure and permeability are strongly affected by sediment clogging and bioturbation processes.

Catchment–scale anthropogenic activities such as agriculture, forestry, logging, mining, and urbanization may contribute to the change in the input of fine sediments into streams (Datry, Lamouroux, Thivin, Descloux, & Baudoin, 2015; Descloux, Datry, Philippe, & Marmonier, 2010; Walling, 2006), potentially leading to fine sediment deposition and clogging in non-transport limited systems. Increasing amounts of fine sediment inputs into freshwaters alter river ecosystem health via the disturbance of key hyporheic processes (Geist & Hawkins, 2016; Lummer, Auerswald, & Geist, 2016; Mueller, Pander, Wild, Lueders, & Geist, 2013; Rehg, Packman, & Ren, 2005). Streambed clogging occurs due to excessive infiltration and accumulation of fine sediments, causing physical alterations of the streambed through the occupation of pore spaces in the sediment matrix (Brunke, 1999; Grischek & Bartak, 2016; Rehg et al., 2005; Schälchli, 1992). Clogging causes a reduction in surface and subsurface water exchange and hence affects various key hyporheic processes such as nutrient exchange, ammonification, and oxygenated water supply necessary for the healthy ecological functioning of freshwater systems (Boulton et al., 1998). Clogging occurs progressively over time as fine sediments infiltrate and cause further deposition, gravitational settling by straining and advection by downwelling pore waters (Casas-Mulet, Alfredsen, McCluskey, & Stewardson, 2017; Casas-Mulet, Lakhanpal, & Stewardson, 2018; Stewardson et al., 2016). Clogging reduces sediment permeability by physically blocking the sediment pores and hindering their ability to transmit fluid (Fox, Packman, Boano, Phillips, & Arnon, 2018; Jin et al., 2019; Pholkern et al., 2015; Reddi, Xiao, Hajra, & Lee, 2005). Consequently, a reduction in hyporheic exchange can be expected, potentially affecting overall river ecosystem function (Allen, 1995; Brunke & Gonser, 1999; Fetzer, Holzner, Plötze, & Furrer, 2017; Packman & Brooks, 2000).

Bioturbation refers to all sediment transport processes produced by the feeding and burrowing of living organisms that affect the physical structure of sediment (Kristensen et al., 2012; Mermillod-Blondin & Rosenberg, 2006; Meysman, Middelburg, & Heip, 2006; Wilkinson, Richards, & Humphreys, 2009). Specifically, invertebrate bioturbation activities such as burrowing, feeding, and excretion have been reported to influence sediment properties in freshwater streambeds (Lewandowski et al., 2019; Nogaro et al., 2006). There are five major groups of bioturbating organisms with respect to their function in the ecosystem. These include biodiffusers, whose activity on the surface results in random diffusion of particles; upward conveyors, which are vertically oriented where the ingestion and egestion move the sediment vertically upwards; downward conveyors, which are oriented vertically, making the sediment particles transport vertically downwards; regenerators, which relocate the sediment particles and create open burrows; and gallery diffusers, which are bioirrigators that create elaborate burrows of tubes interconnected by biotic activity (Mermillod-Blondin & Rosenberg, 2006; Nogaro et al., 2006). Upward conveyors (e.g., Oligochaeta) can reduce the clogging of river beds by burrowing and recirculating the streambed sediment through the creation of galleries (Boeker, Lueders, Mueller, Pander, & Geist, 2016; Nogaro et al., 2007; Roche et al., 2016; Work, Moore, & Reible, 2002). As burrowing and sediment recirculation occur, the increased porosity may lead to a potential increase in permeability, which may promote local oxygenated conditions and lead to hyporheic exchange (Boulton, Datry, Kasahara, Mutz, & Stanford, 2010; Cardenas, Wilson, & Zlotnik, 2004; Wagner & Bretschko, 2002). Bioturbation activity, therefore, can mitigate the effect of clogging by reworking the sediment and preventing fine clay particles from settling (Boulton, Stibbe, Grimm, & Fisher, 1991; Brunke & Gonser, 1999; Ciutat, Anschutz, Gerino, & Boudou, 2005). Through quantitative analysis, Song, Chen, and Cheng (2010) show that bioturbation creates or enlarges pores in clogged beds, further enhancing hydraulic conductivity in streambeds. While it is known that bioturbation activity may help restore hyporheic exchange by modifying the physical properties of streambed sediments (Mermillod-Blondin et al., 2004; Mermillod-Blondin, Gaudet, Gérino, Desrosiers, & Creuzé des Châtelliers, 2003), recent studies illustrate that the role of faunal bioturbation to river functioning may be underestimated (Boeker et al., 2016). Overall, there is little foundational knowledge of the type and magnitude of their effects on streambed sediment structure, essential to understanding the overall implications of bioturbation on river ecosystem functioning at different spatio-temporal scales (Shrivastava, Stewardson, & Arora, 2020). This limited understanding is partly due to the lack of a standard methodology to study the impact of bioturbation on streambed porosity and potential permeability.

Novel technologies such as X-ray micro-computed tomography (micro-CT) provide a promising approach to investigating bioturbation activity in freshwater systems. Micro-CT has helped explore bioturbation networks through fossils in rocks and sedimentary structures (Albani et al., 2010; Baniak et al., 2014; El Albani et al., 2019). Medical CT

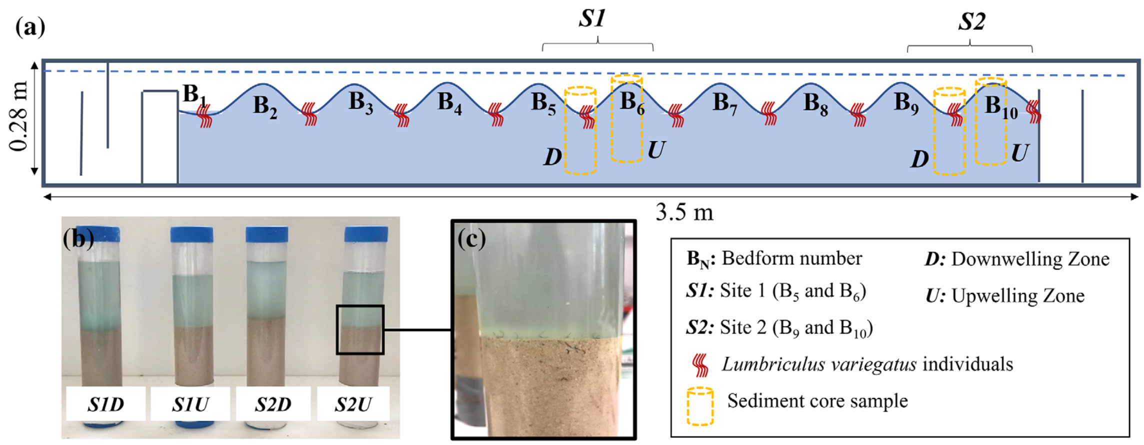

FIGURE 1 Schematic diagram illustrating the different locations of the core samples collected along the experimental flume (a). The images depict the four core samples just after collection and before micro-CT scanning (b), and a zoom in one of the cores to illustrate the top clogged sediment layers (c) [Color figure can be viewed at wileyonlinelibrary.com]

instruments have also been used in marine biology to understand benthic structures (Dufour et al., 2005; Michaud et al., 2003; Rosenberg, Grémare, Duchêne, Davey, & Frank, 2008). Works by Rosenberg, Davey, Gunnarsson, Norling, & Frank, 2007, Mazik, Curtis, Fagan, Taft, & Elliott, 2008, Pennafirme et al., 2019, and Chirol et al., 2021 show how similar technology is used to understand marine sediment processes. However, surprisingly, this technology has never been used in dynamic freshwater environments to assess the influence of living freshwater invertebrates on streambed porosity and potential permeability.

In this study, we aim to develop a novel methodology to investigate the effects of bioturbation activity by freshwater macroinvertebrates in clogged uniform streambed sediments by the use of micro-CT technology as a proof of concept. Through a recirculatory laboratory flume setup, we investigate how network galleries created by *Lumbriculus variegatus*, used as a bioturbator model species, affect sediment structure. We use the developed methodology to address the following specific objectives:

1. To identify and characterise the distribution of bioturbation activity along a clogged sandy bedform streambed, focusing on downwelling vs. upwelling areas.
2. To quantify the extent of gallery networks (or porosity, as a proxy for permeability) created by bioturbation activity, focusing on orientation, depth and volume in downwelling and upwelling areas of the streambed.

2 | MATERIALS AND METHODS

2.1 | Experimental setup

We used a recirculating perspex flume located at the Sexton Ecohydraulics Laboratory at the University of Melbourne. The flume, of dimensions 3.5 m (length, L) × 0.2 m (width, W) × 0.3 m (height, H) (Figure 1) and 0.004 gradient, was attached to a 40–112/23-T485 centrifugal pump (Regent Pumps Pty Ltd.) with a 3-phase motor pump. While disconnected from the pump, we filled the water flume with 0.25 m deep triple-washed sand (average grain size 0.2 mm) layered in sets of 0.5 m batches to avoid air bubbles from getting trapped in the sandy bed. We then proceeded to release a continuous flow of water for several hours to wash out the sand's impurities and dispose of the remaining water. Sandy bedforms with a height of 0.03 m and wavelength of 0.25 m were manually formed along the length of the flume, and the recirculating system was re-connected, maintaining flow rates of 0.0011 $m^3\ s^{-1}$. The system was clogged with clay (average grain size of 0.002 mm) at 1660 $g.m^{-3}$ concentration using a liquid injection at the downstream end of the running recirculatory flume.

Once the system was clogged (after over 48 h), the pumps were shut down, and 10,000 individuals m^{-2} of *L. variegatus* were added to the flume. *L. variegatus* are categorized as upward conveyors as they burrow their heads into the sediment for feeding purposes and eject faecal pellets at the sediment–water interface with the posterior ends (Nogaro et al., 2009). They are typically 0.02–0.05 m long and occasionally can go up to 0.15 m (McCall & Fisher, 1980; Tevesz & McCall, 1985). The chosen population density was based on the natural densities found in lakes and streams with 6,000–8,000 individuals. m^{-2} (Davis, 1974, Mason, Mattson, & Epler, 1994, Work et al., 2002). The bioturbators were left to settle in no-flow condition for 48 h. Afterward, the pump was restarted. The flow rate was increased gradually up to the 0.0011 m^3s^{-1} mark and left running for 12 days, during which the bioturbators were monitored regularly. A single dose of 50 ml of tropical fish food in 1:1 ratio was added in a slurry form and distributed evenly along the flume bedform using a pipette to provide the initial organic matter content in the recirculatory system.

2.2 | Sediment samples

We used cores to sample four locations along the flume, including two sets of each the crest and the troughs of two bedforms located at the upstream (Site 1) and downstream (Site 2) ends of the flume. Each trough and crest of the bedforms represented downwelling (D) and upwelling zones (U) of potential hyporheic exchange (Figure 1). We assumed differences between samples would be encountered as higher bioturbation activity would concentrate in oxygen-richer downwelling zones (Boulton et al., 2010; Hendricks, 1993; Stanford & Ward, 1988). On day 12, saturated core samples were collected using cylinders of 0.3 m depth and 0.05 m diameter for micro-CT scanning. The sediment core samplers were pushed down into the sediment, sealed at the top with a lid, and slowly retrieved upward vertically. The cylinders were sealed at the bottom while in the water using the bottom lid to avoid bubbles forming in the sediment core. The samples were then externally sealed and sent for X-ray analysis. All samples were analysed to a depth of ~0.02 m from the surface of the sediment–water interface, each with a volume of 3.92×10^{-5} m^3.

2.3 | Micro-computed tomography scanning

We performed micro-CT scanning on the four collected samples with a Phoenix Nanotom M (Waygate Technologies) operated using XS control and the Phoenix Datos|x acquisition software. Samples were mounted on the micro-CT stage and positioned vertically to focus on the sediment–water interface (Figure 1). A resolution of 20 μm was achieved, focusing on the micro-CT detector region 0.048-m height and 0.061-m wide. Samples were scanned for 10 minutes (timing = 500 ms, av = 1, skip =0) at 105 kV and 380 μA, collecting 1,199 X-ray projections of each sample through 360° of rotation. A 0.25 mm Cu filter was placed in the collimator on the X-ray source to prevent oversaturation of the X-ray detector and pre-harden the X-ray to help prevent beam hardening effects. After trial and error, these settings were chosen to give the optimal resolution to differentiate burrows from the fine sediment in the core samples.

Volume reconstruction of the micro-CT data was performed using Phoenix Datos|x reconstruction software and data were exported as 16-bit volume files. Volume data was processed using Avizo and the XFiber extension (Thermo Fisher Scientific). Burrow formation due to bioturbation activity was segmented using a tool that correlates cylinders of specified diameter and length within areas of low density (pore space) in the CT dataset. A correlation and orientation field was generated for the specified cylinder size, and the centrelines of objects within the correlation field were segmented. Collected data points along each segment (in this case, separated burrows) detailed various parameters, such as their curved length (total length along the centreline of the burrow) and chord length (distance of a straight line between endpoints of a burrow). The volume of burrows was then segmented separately by dilating the centreline of each burrow to a cylindrical volume encompassing the burrow's diameter and pore space within the CT data. A threshold of darker gray-scale values within the histogram of CT data was used to segment the pore space within the burrows structure. Assuming the structure of a burrow is approximate to that of a cylinder, the total volume (V_b) of the segmented burrows was used in conjunction with their curved lengths (L_c) to determine an average burrow diameter (ϕ_b) using Equation 1.

$$\varphi\phi_b = 2\sqrt{\left(\frac{V_b}{L_c\pi}\right)} \quad (1)$$

2.4 | Burrow analysis

The burrow analysis was performed to quantify the depth and spatial extent of the burrows within each sample. The location of burrow segments was characterised using bounding boxes (i.e., the smallest box that could fit the burrows). The edge of a bounding box in *x*-, *y*-, and *z*-directions, along with the size of the bounding box (D_x, D_y, and D_z), was obtained using Avizo. The edge of the bounding box gives the initial datum to reference a burrow location in the given orientations along *x*-, *y*-, or *z*-axes. Half of the bounding box size is added to provide the geographic centre of a burrow segment. Global centres were corrected relative to the global datums in *x*-, *y*-, and *z*-axes, giving a global centre for each burrow segment. The range in vertical depth a burrow segment traverses was then calculated using Equations 2 and 3, with Equation 2 giving the uppermost boundary (DU) of the burrowing depth and Equation 3 giving the lower boundary (DL) of the burrowing depth.

$$DU = C_z - 1/2D_z \quad (2)$$

$$DL = C_z + 1/2D_z \quad (3)$$

where, C_z is the global *Z* centre of a burrow segment along *z*-axis and DZ is the size of the segment's bounding box along *z*-axis. A similar pair of equations can be defined for the lateral centre positions of a given gallery bounding box in *x*- and *y*-planes. Barycentres were also calculated using the Label Analysis feature of the Avizo software (Thermo Fisher Scientific). The barycentre is the centre of mass of a given segmented gallery.

Furthermore, a tortuosity factor (*T*) was defined using Equation 4, which describes curvature in a burrow segment by dividing its curved length (L_c, the length along the centreline through a burrow) by its chord length (*L*, the distance in a straight line between the two ends of a burrow) as follows:

$$T = L_c/L \quad (4)$$

For analysis purposes, burrow tortuosity was binned into ranges from ≥1.5, 1.49–1.3, 1.3–1.2, 1.19–1.1, and < 1.1, where higher values of *T* represent more tortuous burrows with more curvature, twists, and turns. Another parameter given by Avizo's label analysis, TensorZZ, defines the outer product of unit vectors representing the orientation of segments, weighted by the corresponding segment length *L*, and normalised to have a unit trace. It is an indicator of the vertical or horizontal preference of the segments. The TensorZZ values for segmented burrows were binned into ≥0.6, 0.59–0.4, 0.39–0.21,

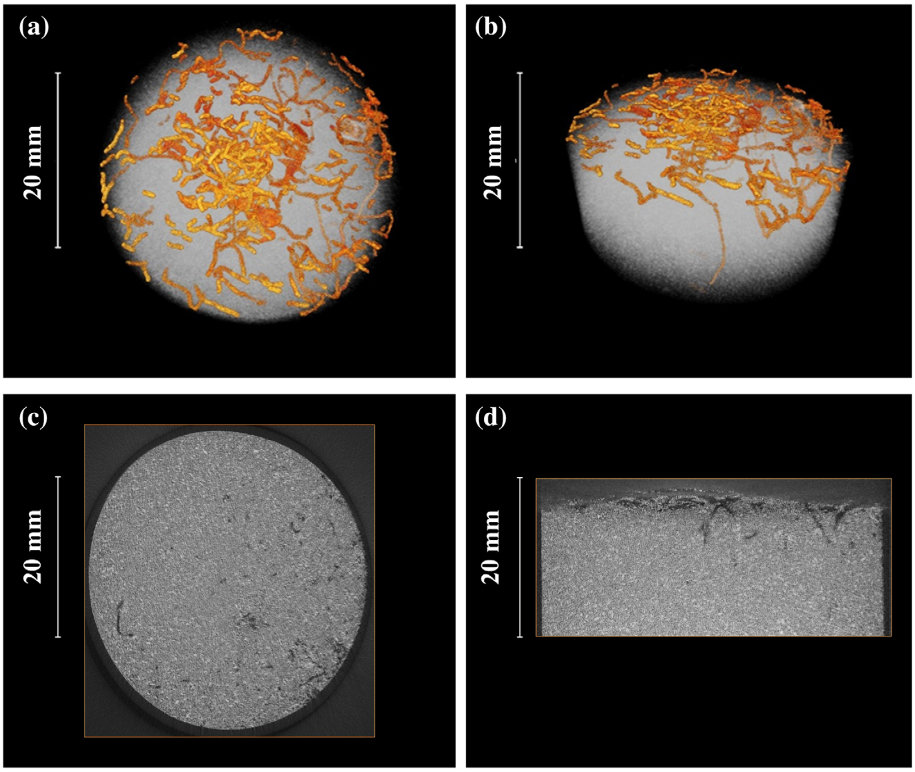

FIGURE 2 X-Ray imagery outcomes of the core sample collected at site S2U showing the galleries created by the bioturbators. They illustrate three-dimensional (a) side and (b) top views, and two-dimensional (c) top and (d) side views of the core sample. Note that the orange segments in panels (a) and (b) are the burrows created by the bioturbators, and the gray material in panels (c) and (d) represents the clogged sand sediment with burrows [Color figure can be viewed at wileyonlinelibrary.com]

and ≤ 0.2, where larger values represent more vertically oriented burrows and lower values horizontally oriented burrows.

3 | RESULTS

3.1 | Galleries and individual burrow visualization

The results from X-ray analysis of the 0.03-m deep sediment core segments collected post-rendering illustrated the distribution of galleried networks created by the bioturbators along, across, and at depth in the bedforms (Figure 2). Many of the galleries appear nearby the sediment–water interface, with some individual burrows branching off to access deeper sediments. These outputs were further processed and used to identify and differentiate between individual burrows and analyse them further. The results of these analyses are presented in the following sections.

3.2 | Gallery distribution in upwelling and downwelling zones

Most of the galleries were concentrated at the surface layers of the sediment, with a few burrows extending vertically deeper than 0.02 m in the downwelling zones (Figure 3, see also Appendix S1 and S2 animations). Galleries were concentrated in shallower sediments, especially in upwelling zones. In S1U and S2U we observed an increase in gallery densities at the surface layers, and both sites illustrated higher bioturbation activity at 0.01–0.015-m sediment depth. Some bioturbation activity at deeper sediment levels beyond 0.015-m depth was observed in the downwelling zones S1D and S2D (Figure 3).

3.3 | Galleries numbers, size, and occupation

The average radius of the burrows was 0.21 mm, with an average curved length of 9.98 mm across all sites. The volume of galleries per unit area was 0.03–0.1 mm for S1D, S1U, and S2D, with a significantly higher volume-to-area ratio in S2U (Table 1).

3.4 | Spatial burrow distribution

The distribution of the bioturbator galleries in depth or vertical *z*-axis (used to plot burrow locations with centres and barycentres in the respective direction, based on Equations 2–4) illustrates an overall pattern of shorter galleries concentrated near the water surface with some site and zone differences (Figure 4). At Site 1 (Figure 4a,b), shorter burrows were observed in the downwelling zone. The upwelling zone showed a gradual increase in length with depth. In S1D, galleries went down to a depth of 0.018 m, with longer galleries concentrated between 0.01–0.015 m. In S1U, galleries were found down to 0.015 m, with longer galleries at 0.005–0.012 m. In both zones of Site 2, longer galleries were concentrated at a depth of 0.005–0.015 m, suggesting a potential boundary effect from the downstream end of the flume.

Lateral distribution showed a fairly uniform scatter of galleries with no trend in horizontal preferential alignment along *x*- and *y*-

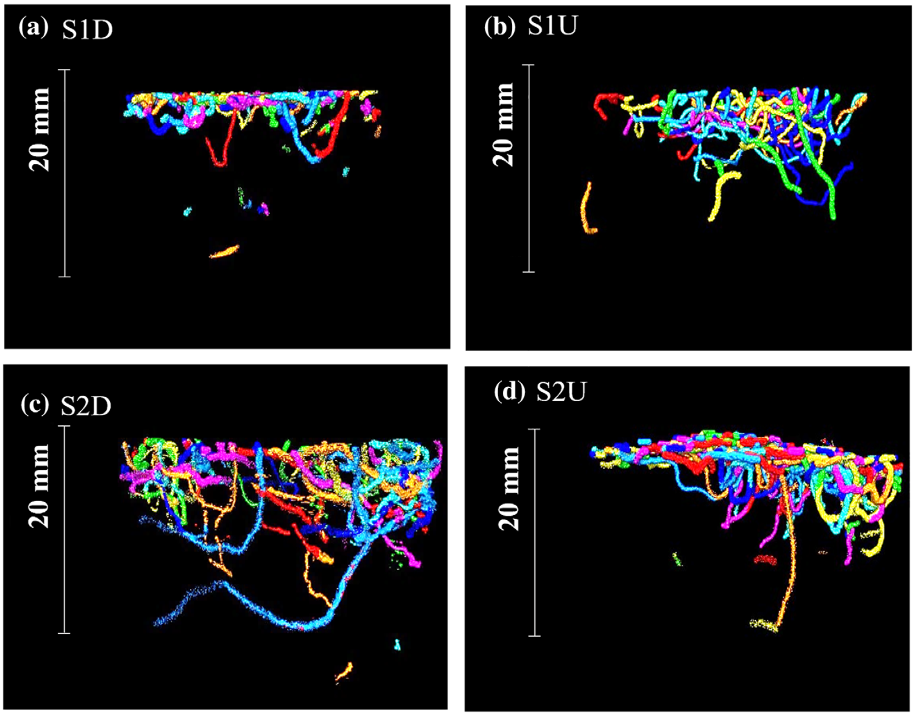

FIGURE 3 Side-view images of individually segmented burrows in each of the four core samples (a) S1D: Site 1, downwelling zone; (b) S1U: Site 1, upwelling zone; (c) S2D: Site 2, downwelling zone; and (d) S2U: Site 2, upwelling zone. Note that each segment is represented by a different color [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Results of the gallery analysis obtained from the Micro CT-scans performed in all four core samples (S1D, S1U, S2D, S2U). Presented statistics include gallery numbers, total volume occupied per gallery, volume ratio (total volume of galleries to volume of sample collected), volume of galleries to area ratio (total volume of galleries to total area of sample collected), average chord length, average curved length, and average radius of galleries

Site	Number of galleries	Total volume (mm^3)	Average gallery volume (mm^3)	Volume ratio	Volume/area ratio (mm)	Average chord length (mm)	Average curved length (mm)	Average gallery radius (mm)
S1D	144	105.67	0.77	0.0026	0.0336	2.96	3.67	0.24
S1U	67	135.27	2.09	0.0034	0.0431	8.49	15.32	0.21
S2D	50	45.95	0.73	0.0011	0.0146	8.91	16.34	0.13
S2U	367	370.41	1	0.0094	0.1179	3.46	4.59	0.27

planes (Figure S1 graphical abstract provided for lateral distribution), hence showing no edge effect of the sampling technique adopted in the experimental procedure.

3.5 | Gallery orientation and segment turns

TensorZZ values are an indicator of relative burrow orientation, with higher values (closer to 1) indicating a more vertical burrow structure and lower values (closer to 0) indicating horizontal burrows. Overall, TensorZZ values were lower than 0.4 (Table 2), suggesting that most burrows were preferentially oriented in the horizontal plane with little differentiation in gallery orientation and alignment between Sites 1 and 2.

Tortuosity values indicate the shape of the burrows created by the bioturbators, indicating whether turns or curvature occur along a burrow segment. Higher tortuosity values (greater than 1.5) indicate more turns or significant curvature, and lower values indicate straight burrows with little to no curvature (Table 3). In S1D and S2U, more than 80% of galleries presented tortuosities of less than 1.3, while in S2U and S1D larger percentages of galleries presented >1.3 tortuosity values.

4 | DISCUSSION

This study presents a proof of concept of the potential for micro-CT imaging to identify, characterise and quantify the bioturbation activity of macroinvertebrates in clogged streambeds. We used X-ray CT to produce a 3D segmentation of the gallery structures created by *L. variegatus* in the sediment structure of a flow-through flume streambed. We observed high bioturbation activity in the top layers of the sediment, suggesting a potential increase in porosity at the water–sediment interface. Only a few galleries were observed at depths greater than 0.02 m, and mostly in downwelling areas.

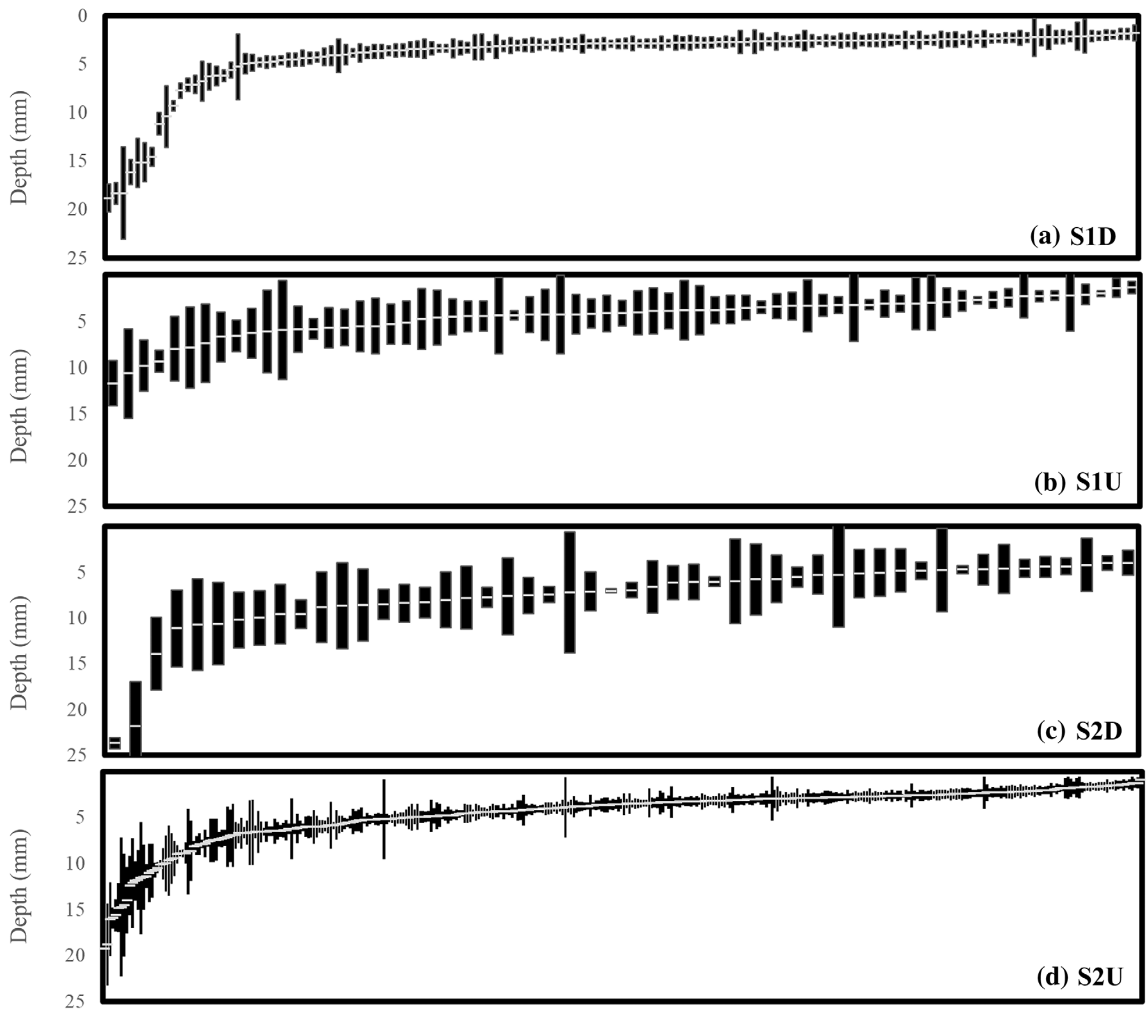

FIGURE 4 Vertical distribution of the galleries along *z*-axis for (a) S1D: Site 1, downwelling zone, (b) S1U: Site 1, upwelling zone, (c) S2D: Site 2, downwelling zone, and (d) S2U: Site 2, upwelling zone. Note the black bars show the length of the segments and the gray lines indicate the CentreZ for each of the segments

TABLE 2 Percentage of burrows with a TensorZZ values of the range between ≤0.2 and ≥0.6 for the four collected samples

Range	S1D (%)	S1U (%)	S2D (%)	S2U (%)
≥ 0.6	5.55	7.46	8	6.53
0.59–0.4	8.33	14.92	22	8.71
0.39–0.21	20.83	47.76	48	11.17
≤ 0.2	65.27	29.85	22	73.56

TABLE 3 Percentage of burrows with tortuosity values of range between <1.1 and ≥1.5 for the four collected samples

Range	S1D (%)	S1U (%)	S2D (%)	S2U (%)
≥ 1.5	8.33	38.8	42	9.26
1.49–1.3	11.11	19.4	16	8.17
1.29–1.2	11.8	13.43	12	8.99
1.19–1.1	19.44	8.955	18	18.52
< 1.1	49.3	19.4	12	55.04

Micro-CT scanning is used here for the first time in a dynamic freshwater context, proving to potentially be an effective approach to map bioturbator galleries and understand their role in the physical structure of freshwater streambeds. Although previous studies by Pigneret et al., 2016 and Mermillod-Blondin et al., 2018 focused on using micro-CT to understand the impact of bioturbation on sediment transport in freshwater systems, our study focusses in a deeper understanding of the physical structures of the bioturbator galleries and the physical impact of the extensive galleried networks created by the organisms in the sub-surface sediment. Our observations that most burrowing activity was concentrated just below the sediment–water interface, at approximately 0.02 m depth in the streambed, aligns with the observations in Nogaro et al. (2009) and Statzner (2012). The spatial distribution of bioturbation activity varied along the flume streambed length, with a potential boundary effect from the downstream end of the flume. Overall, however, downwelling zones showed higher burrow count potentially due to the comparatively higher aerated and nutrient-rich water in such areas, which promoted hydraulic exchange (Allen, 1995; Ciutat et al., 2005), and higher bioturbation activity (Blankson, Deb Adhikary, & Klerks, 2017; De Backer et al., 2011; Ouellette et al., 2004). A strict pattern of deeper burrowing activity in the downwelling zone was, however, not clear from the present study, needing studies with larger sample sizes and more replication to test this hypothesis statistically.

In terms of quantification, the burrow analysis showed that more than 60% of the galleries in all four samples were significantly aligned in the horizontal plane instead of vertically. Overall, most galleries presented straight instead of U-shapes and displayed low tortuosity values (approximately between 1.1–1.5). Such findings are consistent with what is expected of *L. variegatus*, an upward conveyor bioturbator type that feeds head down (McCall & Fisher, 1980; Mermillod-Blondin & Rosenberg, 2006; Nogaro et al., 2006). The ratio volume/area occupied by galleries was extensive and provided the basis to assume a potential increase in sediment permeability via increased porosity, which is particularly important to help offset the effects of streambed clogging (Mao et al., 2020). Roche et al., 2016 showed that the burrows were biased to the top 0.01 m of the bed, leaving large areas of the subsurface unaltered, with very rare activity in the deeper sections of the core. These observations are well supported by this study both in terms of depth and behaviour of the bioturbators and the galleries they formed, showing that very little sediment mixing occurred beyond the top 0.01 m of the sediment–water interface. As much as it is interesting to observe the large differences between sites at upwelling and downwelling zones among the sediment cores, we cannot rule out the possibility of other response variables of the system, such as micro-distribution of organic matter and fine particles ingested by individuals, and bioturbator responses at finer scales, which could have influenced bioturbation activity.

We acknowledge the lack of replicates of our study to strengthen the arguments made above. However, given that this experimental study is a first attempt at using micro-CT Scanning to understand the impact of bioturbation in clogged riverbeds in a dynamic flow context, our results can provide the basis for improvement in future studies. One of the following main issues of our methodological approach was the ongoing bioturbation activity from live individuals during the CT scan: The moving *L. variegatus* created blurriness and noise in the scanned data, which required heavy smoothening to be able to differentiate and identify individual segments for the burrow analysis. Such noise in the scanned data resulting from the movement of live individuals could potentially be overcome by freezing the samples using liquid nitrogen. Such a technique could also help to identify the degree of bioturbation in natural mixed taxon biotic communities. However, the characterization and differentiation of the bioturbators and their structures, along with other elements of coarser nature, can add an element of complexity that may require heavy smoothening of the data. Another major limitation of the use of this technique is the difficulty of distinguishing between bioturbators and their galleries, which can result in a subjective interpretation of whether the less active bioturbators occupy existing galleries and/or others actively continue burrowing and feeding movements resulting in the collapse of parts of the existing galleries and the creation of new ones simultaneously. In addition, our assumption of oxygen being the main factor in promoting bioturbation in the downwelling areas was not supported by in-situ measurements.

Despite the limitations, this study provides valuable insights into how invertebrate bioturbators align their galleried networks and how their activity promotes porosity, potentially increasing permeability and supporting hyporheic exchange by compensating clogged conditions in the sediment structure. Furthermore, our presented methodology is fully transferable and can be used at other spatial scales, including natural streambeds and different bioturbation species. This study suggests we should give the role of bioturbation activity more attention as a tool to maintain hyporheic fluxes in streambeds and support overall river ecosystem function. Such understanding is critical to inform sustainable water management approaches and river restoration practices at multiple spatiotemporal scales (Gilvear, Spray, & Casas-Mulet, 2013; Wohl, Lane, & Wilcox, 2015).

5 | CONCLUSIONS

We provide the first proof of concept of the potential for micro-CT imaging to identify, characterise and quantify the created galleries by macroinvertebrate bioturbation in clogged streambeds. Despite some limitations in the method, our study helps understand the role of bioturbation activity in increased porosity and potentially compensating for excess fine sediment accumulation in streambeds through increased permeability. Furthermore, our methodology is transferable and can be used as a basis for improvement in broader studies, including natural systems and a range of other bioturbator species. A clear understanding of the importance of bioturbation processes in maintaining hyporheic fluxes and supporting river ecosystem function is essential to inform river restoration practices.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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