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Effects of bioirrigation on the spatial and temporal dynamics of oxygen above the sediment–water interface

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Abstract: Burrow ventilation by tube-dwelling benthic animals affects solute exchange between sediments and water by 2 means. Drawing of O$_2$-rich water into the burrow increases O$_2$ availability in the sediment and stimulates biogeochemical and microbial processes, whereas flushing of the burrow creates a 3-dimensional flow field above the burrow, which induces mixing. Previous studies have revealed the role of the diffusive boundary layer (DBL) thickness on the exchange of solutes between the sediment and overlying water. Mapping the O$_2$ gradient within the DBL is a challenging task in the presence of benthic faunal activities. We used a novel lifetime-based laser induced fluorescence ($\tau$LIF) technique that enables unobstructed observations of spatial and temporal O$_2$ dynamics above burrows inhabited by midge larvae (*Chironomus plumosus*). We observed instantaneous plumes of O$_2$-depleted water released from the outlet of the burrows and drawdown of O$_2$-rich water above the inlet caused by peristaltic pumping of *C. plumosus* larvae. Vertical O$_2$ gradients changed dynamically during burrow ventilation relative to in a control tank without animals. The advective transport of O$_2$ above the opening caused by burrow ventilation degraded the O$_2$ concentration gradient. For a range of larvae densities that is frequently observed in ponds and lakes, the advective transport caused by burrow ventilation was the dominant transport mechanism.

Key words: oxygen flux, diffusive boundary layer, bioturbation, sediment-water interface, laser-induced fluorescence, oxygen-sensitive nanobeads
Bioturbation of aquatic sediments by benthic fauna was defined by Kristensen et al. (2012) as the combined effect of particle reworking (sediment movement) and burrow ventilation (water movement). Burrow ventilation by tube-dwelling benthic animals draws O\textsubscript{2}-rich water through a burrow inlet and releases plumes of deoxygenated water through a burrow outlet. The ventilation creates complex 3-dimensional concentration distribution in the sediment (Lewandowski and Hupfer 2005) and 3-dimensional flow fields above the burrow (Morad et al. 2010, Roskosch et al. 2010). The enhanced O\textsubscript{2} flux into the sediment stimulates aerobic respiration and is accompanied by enhanced exchange of solutes between sediment and overlying water (Matisoff and Wang 1998, Mermillod-Blondin et al. 2004, Hölker et al. 2015). The enhancement of sediment O\textsubscript{2} uptake promoted by tube-dwelling benthic animals has been examined in numerous studies (Hargrave 1972, Pelegri and Blackburn 1996, Hansen et al. 1998, Matisoff and Wang 1998, Baranov et al. 2016) and depends on the species present, their abundance, the overlying O\textsubscript{2} concentration, and temperature (Roskosch et al. 2012, Baranov et al. 2016).

Tube-dwelling animals, such as *Chironomus plumosus* larvae, construct U-shaped burrows, some of which can extend up to 15 cm below the surface of the sediment (Granéli 1979). For some benthic animals, such as *Pysgospio elegans*, *C. riparius*, and *Chironomus dorsalis*, the inlet of the tube typically extends a few millimeters above the surrounding sediment. This height is assumed to bypass the low-O\textsubscript{2} concentration region of the boundary layer overlying the sediment–water interface (SWI) (Jørgensen and Revsbech 1985, Pinder 1986, Stief et al. 2005).

The motion of *C. plumosus* that generates burrow ventilation takes the form of undulations of their \(~\sim\)20-mm-long body that travel in the head-to-tail direction as a sinusoidal
wave (Brackenbury 2000). This motion creates 3-dimensional flow fields above the burrow inlet and outlet (Munksby et al. 2002, Morad et al. 2010, Roskosch et al. 2010). In alternating periods of pumping and resting, the larvae draw O$_2$-rich water into the burrow while pumping, absorb and store the O$_2$ in hemoglobin, and use the O$_2$ in the hemoglobin for metabolism during the periods of feeding or resting (Walshe 1950). *Chironomus plumosus* larvae adjust to low O$_2$ conditions by becoming immobile and using the O$_2$ stored in the hemoglobin for up to 9 min (Walshe 1950).

The O$_2$ dynamics during bioturbation have been studied mainly in the sediments, and most observations from above the sediment surface are limited to flow velocities or pointwise concentration measurements. Flow-field observations based on particle image velocimetry (PIV) revealed the existence of large-scale plumes, particularly at the burrow outlets (Morad et al. 2010, Roskosch et al. 2010). The effect of these flows on the O$_2$ concentration distribution and mean vertical concentration gradients have not been investigated.

We used a recently developed lifetime-based laser induced fluorescence (τLIF) technique (Murniati et al. 2016) to analyze the effect of burrow ventilation of *C. plumosus* on the spatial and temporal O$_2$ dynamics above the SWI. The τLIF O$_2$ imaging system enables unobstructed observations of planar O$_2$ concentration dynamics above the SWI with high spatial resolution. We hypothesized that the diffusive boundary layer (DBL), which controls sediment–water fluxes of O$_2$ in the absence of bioirrigation (Lorke and Peeters 2006), becomes gradually degraded with increasing organism density. We analyzed the temporal dynamics of O$_2$ concentration distributions above burrows as a function of organism density and in relation to their ventilation activity. We quantified the resulting mean O$_2$ gradients in the boundary layer and compared them to the nonbioturbated case to investigate the effect of bioturbation on the diffusive
boundary-layer dynamics above the SWI.

METHODS

Experimental setup

We observed the dynamics of near-sediment O₂ distributions around natural burrows of *C. plumosus* in a series of laboratory experiments. We placed the organisms with varying abundance in 3 experimental chambers (tanks; 7 × 7 × 10 cm [length × width × height]), which were filled with 5 cm of sediment and 5 cm water. The sediment surface was slightly inclined (~12°) to permit an unobstructed view of the sediment surface, which was illuminated by the laser light sheet with a horizontally oriented camera. The experimental tanks (T1–T3) contained 3 (T1), 6 (T2), and 12 (T3) larvae corresponding to larval densities of 612, 1224, and 2448 individuals (ind)/m², respectively. An additional tank (T0) without larva provided a nonbioturbated control. During the experiment, all test tanks were closed with airtight transparent lids and kept in a water bath (40 × 30 × 22 cm) that maintained the temperature in the tanks at 21 ± 0.8°C. Except for the periods of laser illumination, we kept the tanks in the dark during the entire experiment.

Before placing the tanks in the water bath, we identified active burrows in each tank by placing a fast-response O₂ sensor (FirestingO₂; Pyro science GmbH, ) close to the opening of each burrow. We observed the unobstructed spatial and temporal dynamics of O₂ concentrations around the selected burrow in- and outlets with the aid of planar (2-dimensional) lifetime-based laser-induced fluorescence (τLIF) measurements and suspended nanoparticles coated with an O₂ indicator (Murniati et al. 2016). The distances from the selected burrow opening to the nearest rigid walls were 1.5, 2.8, and 2.8 cm for T1, T2, and T3, respectively. The field of view used in
T0 was at the center of the tank. We made hourly measurements of ~10-min duration each (300 concentration images) in all 4 tanks for a period of ~15 h (Table 1). We added 0.35 mL of stock solution containing the O2-indicator-coated nanobeads (equal to 0.24% of the volume of water in the tank) to each tank. The O2 concentration in all tanks was adjusted sequentially to 80%-air saturation (220 µmol/L at 21°C) by purging with N2 or reaerating the water while mixing the O2 indicator nanobeads in the tank. Thereafter, the tanks were closed. We report measurement times as relative time (hh:mm) after closing of the tanks. Before the measurements, we conducted calibration experiments for converting fluorescence lifetime to O2 concentration by varying the O2 concentration in a tank (7 × 7 × 10 cm) with sediment but without animals. The same amount of O2 indicator (0.24% of the volume of water) was used. The O2 concentration was adjusted by purging the water with N2 gas or atmospheric air. The calibration experiment was repeated after 30 h. During the final calibration, sodium sulfite (Na2SO3) was added in surplus to obtain an O2 concentration of 0 µmol/L. In total, we observed 17 O2 concentrations ranging between 0 and 272 µmol/L. For each of these O2 concentrations, we collected 30 to 50 image pairs.

**Sediment and organism preparation**

We collected natural sediments from a small harbor at the Rhine River in Germersheim, Germany (lat 49.221717 N, long 8.382687 E), on 1 June 2016. We used an Ekman grab sampler to collect the upper 20 cm of the sediment at a location ~3 m from the shore at a water depth of 3 m. We homogenized the sediment and passed it through a 1-mm sieve to remove debris and a 300-µm sieve to remove small organisms. We stored the sieved sediments in beakers at 4°C for 1 wk. Three days before introducing the larvae, we filled the test tanks with sediment and added
tap water up to the rim.  

We used cultured *C. plumosus* larvae (Amtrax Live Premium; Kölle Zoo-Karlsruhe, ) for the experiments. Cultured larvae have similar behavior to freshly collected organisms in bioirrigation experiments (Baranov et al. 2016). We selected 4th-instar larvae based on body length (18–28 mm; Hilsenhoff 1966). We left the larvae in the test tank for 2 d to acclimatize and construct burrows. During this time, the water in the tanks was aerated continuously, and the tanks were covered by an insect net to prevent the escape of emerged adults. We counted the larvae remaining in the tanks at the end of the experiments.

τLIF setup

We measured O₂ concentration by evaluating the fluorescence lifetime of Platinum(II)-5,10,15,20-tetrakis-(2,3,4,5,6-pentafluorphenyl)-porphyrin (PtTFPP) coated on nanobeads (diameter <500 nm) which were suspended in the water of all test tanks. We used the O₂ indicator nanobeads (PtTFPP) from the same batch as a previous study (Murniati et al. 2016). Fluorescence excitation was provided by short pulses from a 405-nm diode laser with a Powell lens, which expanded the laser beam into a 1-mm thick planar light sheet. We accumulated the fluorescence intensities within 2 distinct time windows following the excitation pulses for a number of individual excitations on separate images recorded by 14-bit CCD Camera (PCO.1600; PCO AG, ) with an electronic shutter. A sequencer unit generated the laser pulses and controlled the camera shutter. A detailed description of the fluorophores and the measurement system was given by Murniati et al. (2016).

We fixed the laser on a frame and illuminated the selected sediment surfaces from above. We placed the camera on a linear actuator plate to adjust the focus and mounted it on a vertically
movable frame perpendicular to the light sheet. The setup enabled us to position the light sheet across a burrow opening and to adjust the focus of the camera to the position of the light sheet. We optimized the operational parameters of the τLIF measurements during preliminary tests to provide sufficient accuracy of individual O₂ concentration measurements and a fast sampling rate (see Murniati et al. 2016 for a detailed description of these parameters and their effects on measurements performance). We used a duration of fluorescence excitation of 25 µs, an equal exposure duration of 16 µs in both time windows for fluorescence intensity measurements, and a delay of 10 µs between both windows. We accumulated a train of 17,000 light pulses on each intensity image, resulting in a 2-s sampling interval for an integrated O₂-concentration image. The spatial resolution of the concentration estimates was 12 µm within a field of view with the dimensions of 14.4 × 19.2 mm.

τLIF data processing

We removed outliers in the recorded fluorescence intensity images by applying the phase-space method, an algorithm frequently applied for detecting spikes in acoustic Doppler velocimetry data (Goring and Nikora 2002). We replaced the outliers with values calculated from a cubic polynomial fitted to the remaining data. We smoothed the fluorescence lifetime distributions with a low-pass Gaussian filter with a 7 × 7-pixel (84 × 84 µm) window size for noise reduction. We obtained final O₂ concentration estimates by fitting the 2-site model (Sacksteder et al. 1993) to the lifetime estimates obtained from the calibration measurement. Fluorescence intensity decreased significantly during the experiments because of sedimentation or filtration of the fluorophore particles or photo-bleaching, but the pre- and post-calibration experiments provided consistent results (Fig. S1). Based on the performance estimates described
by Murniati et al. (2016), we removed all measurement sequences for which the mean image intensity was <1100, which is close to the camera noise (Fig. S2). This process led to a reduction available measurement sequences for subsequent analysis (Table 1).

178

179 **SWI detection and vertical O₂ profiles**

180 Bottom reflection of the laser light and high bead concentration resulting from sedimentation caused a pronounced fluorescence intensity maximum at the SWI. The vertical position of the SWI within the laser light sheet was detected as the local maximum in fluorescence intensity. We defined the SWI as 0 ordinate (0 vertical axis) and moved all pixel columns accordingly. In some measurements, the SWI in the laser sheet was partly blocked by sediment in front of the light sheet because of sediment reworking by the chironomids. In these cases, we could not observe the lower part (0.16–0.45 mm) of the vertical O₂ profile because of a combination of inclined sediment surface, thickness of the light sheet, and sedimentation of beads, so we extended the measurements to the SWI to define the O₂ concentration at the SWI ($C₀$) (Fig. S3). We defined the upper limit of the diffusive boundary layer ($C∞$) as the intersection of the linear gradient of the O₂ concentration with the bulk-water O₂ concentration (cf. Fig. S4), calculated as the mean concentration in the extrapolated vertical profiles.

192

193 **RESULTS**

194 **Sediment reworking**

195 Chironomid larvae started burrow construction immediately after they were placed in the test tanks. One day after introducing larvae to the tanks, all organisms were in burrows. The sediment surface in all tanks was initially inclined and carefully trimmed to achieve a relatively
smooth topography to ensure an unobstructed view on the illuminated plane (Fig. S3A). However, small-scale variation in the sediment topography is inevitable. Thus, locally elevated sediment surface between the illuminated plane and the camera occasionally blocked the camera’s view of the actual sediment–water interface at the plane of interest. The treatment of such blocked area is explained in Fig. S3B.

In the tanks with chironomids, the sediment topography within the field of view changed throughout the measurement period (Fig. 2A–C). In T0, the sediment surface remained similar to its initial condition (<0.2-mm vertical variations), but in the other tanks, vertical variation increased to as much as 4 mm. Temporal variation in the sediment surface was lower at the outlet (Fig. 2B) than around the inlet (Fig. 2A). These observations suggested that the modifications around the inlet were caused by direct sediment reworking of the chironomids, whereas around the outlet may have been caused by burrow ventilation and sedimentation of resuspended particles. In T3, a rapid change of the sediment topography around a burrow opening occurred between 10:01 h and 11:20 h). Within 1 h, a chimney—a typical structure for an inlet—was formed (dotted line in Fig. 2C), and we observed a change of the O₂ concentration structure above the opening from predominantly release of deoxygenated plumes to predominantly drawdown of O₂-rich water (see further explanation in O₂ dynamics below). The altered O₂ structure above the opening might have been caused by undulation of the larva in the burrow in reverse direction or by a change in the orientation of the larva within the burrow.

O₂ concentration dynamics above burrows

Burrow ventilation was associated with the release of upward-propagating pulses of water with low O₂ concentration (Fig. 3A–C). The concentration in the initial plume of a pulse
sequence could be as low as 10% saturation, but increased over time in subsequent pulses, indicating flushing of the burrow with water having higher O₂ concentration. The individual plumes propagated upward and developed into a mushroom-like shape while spreading horizontally and entraining ambient water (Fig. 3C). The horizontal extent of the plumes was typically ~4 mm, whereas the vertical dimension often exceeded the field of view (>8 mm).

Particularly during periods of sustained pumping activity (e.g., at low O₂ concentration in T3), the upward propagating plumes drove coherent vortices, with outward flow at the top of the plume and inward-directed flow at the bottom. With these vortices, which had dimensions exceeding the size of the field of view, water from heights >1 cm above the SWI with high O₂ concentration was continuously transported toward the sediment surface.

Visual analysis of subsequently observed concentration distributions indicated that plume velocities were mostly in the range of 0.6 to 2.1 mm/s with a maximum value of 4.8 mm/s (T2, +15 h). In T2 and T3, we occasionally observed packages of water moving into the field of view from the side. These packages probably were advected by larger-scale flow structures from neighboring burrow outlets.

Above the burrow inlet in T1, the O₂ concentration distribution was less dynamic. During pumping events, the bulk O₂ was drawn into the burrow where the maximum drawdown occurred in the center of the opening and extended up to 2 mm above the SWI (Fig. 3E). Within a radius of 1 to 3 mm around the burrow inlet, the O₂ concentration was highly affected by the drawdown, whereas at larger distances from the opening the concentration was less dynamic and more homogeneous. At the end of a pumping period (a sequence of pulses) or during periods of rest, we occasionally observed the release of small amounts of low-O₂ water from the inlet (Fig. 3F). We attribute this rather unexpected observation to small currents produced by movements of
chironomids in the burrow during feeding or net construction. This interesting behavior was also found above the burrow opening in T3, where the observed O2 structure above the opening was variable. In some sequences, the O2 drawdown into the burrow was more dominant than the release of deoxygenated plumes. The peculiar O2 structures observed in T3 might be related to sediment reworking by the larva during chimney construction, as indicated by a significant increase of the sediment surface around the burrow opening (cf. Fig. 2C). The O2 concentration distribution in the tank without chironomids (T0) was horizontally more homogenous and did not show significant short-term dynamics. In contrast to the observations above chironomid burrows, the O2 gradients were restricted to a thin layer above the SWI.

**Temporal dynamics of ventilation activity**

Burrow ventilation was characterized by pulses of flow above openings and resulted in distinct O2 structures above the burrow inlet (cf. Fig. 3D, E, Video V1) and outlet (cf. Fig. 3A–C, Video V2). Based on visual inspection of the concentration video sequences, we categorized the observations into ventilation and resting periods. We observed pulses of flow during individual ventilation events. The duration of active ventilation in a measurement sequence is the sum of the duration of individual ventilation events. The duration and frequency of individual ventilation events and the total ventilation duration are summarized in Table 2. During an individual ventilation event, pumping pulses were created approximately every 8 s. In T1 and T2, an average of 3 ventilation/pumping events with durations of ~2.5 min were observed during the 10-min measurement sequences, and the larvae spent ~50% of the time engaged in active burrow ventilation. In both tanks, ventilation activity varied strongly among the measurement sequences. The ventilation activity in T3 was comparable to that in the other 2 tanks for the first
4 h of measurements only, before the duration of the ventilation sequences increased strongly. On average, the duration of the ventilation sequences in T3 was 2× high as in T1 and T3, and the larvae spent nearly 80% of their time on burrow ventilation (Table 2). Uninterrupted ventilation activity was observed during some measurement sequences. The observed differences in pumping activity in T3 probably were related to the low O₂ concentration in this tank. The combination of data from the 3 tanks revealed a decreasing trend of pumping activity with increasing bulk O₂ concentration (Fig. 4). The observed fraction of active venting decreased from nearly 100% at O₂ <10% saturation to ~50% at O₂ saturation around 60%.

**Mean vertical O₂ gradients**

Mean vertical gradients of dissolved O₂ above the SWI were estimated by lateral averaging of the entire field of view (412–1045 pixel columns) and temporal averaging of all concentration images in a measurements sequence (249–436 concentration images). The resulting profiles differed substantially among the 4 tanks and measurement times (Fig. 5A–D). In the control tank, the profiles were characterized by 2 nearly linear sections, with the stronger vertical gradient within the lowest 1 to 3 mm above the SWI (Fig. 5A). Above this concentration boundary layer, we observed a weaker, but persistent vertical gradient that can be attributed to the slow rates of vertical mixing in the stagnant tanks. Both bulk concentration and the O₂ concentration at the SWI decreased over time.

The profiles in T1 (low chironomid density) had a shape similar to those observed in the control tank, but the temporal decrease of O₂ concentration was much less pronounced (Fig. 5B). We attribute this to a potential leakage at the lid of T1 (see below). The mean vertical concentration gradients in T2 and T3 were much weaker (Fig. 5C, D), and in contrast to the
control tank (T0), the gradient in the lower part of the profiles gradually decreased over time. In T3, the concentration dropped below 15 µmol/L after the first 4 h of the experiment and was characterized by a constant weak O₂ gradient over the observed depth range.

**DBL layer fluxes**

In the absence of bioirrigation, sediment O₂ uptake rate is controlled by sediment O₂ demand and the diffusive O₂ flux across the water-side concentration boundary layer. Sediment O₂ uptake rate can be described as the product of a transfer velocity and the concentration gradient (Lorke and Peeters 2006):

\[ F_{DBL} = \frac{D}{\delta_{DBL}} (C_\infty - C_0) \]  

Eq. 1

where \( F_{DBL} \) is the O₂ flux, \( D \) is the diffusion coefficient of O₂ (\( D = 2.4 \times 10^{-5} \) cm²/s at 21°C), \( C_\infty \) is the concentration at the upper end of the DBL, \( \delta_{DBL} \) is the thickness of the DBL, and \( C_0 \) is the O₂ concentration at the SWI. The conceptual basis of Eq. 1 limits its application to T0, where the transport can be considered to be governed by diffusion only. In T0, the observed \( \delta_{DBL} \) increased continuously over time to >3 mm at the end of the experiment (Fig. 6A). The 3-fold increase of \( \delta_{DBL} \) led to a corresponding decrease of the O₂ flux across the SWI from initially 12 to 3 mmol m⁻² d⁻¹. Despite the broad range of temporal variability of \( \delta_{DBL} \) (Fig. 6A) and O₂ concentration (Fig. 5A), the presentation of mean vertical O₂ profiles in a dimensionless form based on \( z/\delta_{DBL} \) vs \( (C-C_0)/(C_\infty-C_0) \) collapsed into a universal shape within the diffusive boundary layer (Fig. 6B). Above the DBL, the O₂ concentration gradient also varied with time in the dimensionless presentation. These variations probably can be attributed to the variability of the weak, large-scale convectively driven mixing in the tank.
O2 consumption and tentative mass balance

We estimated the mean O2 concentration in the water column above the SWI by extrapolating the upper part of the laterally averaged concentration profiles (Fig. 5A–D) to the water surface and subsequent vertical averaging over the entire water column. Within the first 6 h of observations, the mean O2 concentrations in T0 and T1 decreased at a similar rate, whereas they decreased much faster in T2 and T3 (Fig. 7). The concentration in T3 dropped to <15 µmol/L during this time, but it approached rather constant values of ~70 and 150 µmol/L\(^{-1}\) in T1 and T2, respectively. We assume that the unexpected stagnation of the mean O2 concentration in T1 and T2 was caused by leakage and atmospheric aeration at the lids of these tanks. Therefore, we restrict the following analysis of the O2 mass balance to the first 6 h of measurements, where a consistent decrease of O2 concentration could be observed in all tanks.

For T0, we compared the observed rate of decrease of mean O2 concentration with the rate resulting from the O2 flux across the SWI (Eq. 1):

$$C_{DBL}(t_i) = C(t_{i-1}) - \frac{A}{V} F_{DBL}(t_i)(t_i - t_{i-1})$$

Eq. 2

where $C(t_{i-1})$ and $C_{DBL}$ are the initial concentration and the predicted concentration at some later sampling time ($t_i$), respectively. $A$ (50 cm\(^2\)) and $V$ (160 mL) are sediment surface area and volume of water, and $F_{DBL}$ is the flux across the SWI estimated from DBL thickness (Eq. 1, Fig. 6A). The observed decrease of O2 concentration in the water column of T0 was in very good agreement with the concentration estimated from Eq. 2 (Fig. 7), indicating that the DBL fluxes estimated from the vertical concentration gradient were representative for the entire tank and that no other sources or sinks for O2 were present in T0.

To compare the O2 dynamics in the tanks with chironomid larvae to that in T0, we estimated the sediment–water O2 flux, which corresponds to the observed concentration decrease
in the water during the first 6 h of observation \( (F_{dC/dt}; \text{Table 3}) \). The flux was comparable in T0 and T1, but it was increased by factors of 2.2 and 2.5 in T2 and T3, respectively. We estimated the contribution of organism respiration to the observed areal \( \text{O}_2 \) flux in the tanks by multiplying literature-based respiration rates of \( \textit{C. plumosus} \) by larval density in the test tanks (Table 3). Depending on the particular choice of chironomid respiration rate, the observed flux enhancement by burrow ventilation exceeded the respiratory \( \text{O}_2 \) demand of the chironomids by up to a factor of 3.8 (Table 3).

**DISCUSSION**

Our study yielded the first unobstructed observation of the spatial and temporal dynamics of \( \text{O}_2 \) concentration above a sediment–water interface in the presence of infaunal activity. Planar \( \text{O}_2 \) concentration dynamics above the sediment–water interface created by burrow ventilation by midge larvae (\( \textit{C. plumosus} \)) were revealed and enabled visualization of small-scale fluid mechanics generated by activity of benthic organisms. Application of this novel \( \tau \text{LIF} \text{O}_2 \) imaging system demonstrated its ability to resolve benthic DBL dynamics. Thus, it facilitates a more detailed analysis than has been possible in the past of \( \text{O}_2 \) transport mechanisms across the sediment–water interface under complex conditions.

The fluorescence intensity signal decayed during the measurements (Fig. S2), indicating a decrease of nanobead concentration or photo-bleaching of the \( \text{O}_2 \) indicator coating. The filtration efficiency of \( \textit{C. plumosus} \) has been reported to be highest for particles >17 µm in diameter (Walshe 1947). Therefore, we expected that the small (<0.5 µm) \( \text{O}_2 \)-sensing nanobeads would be affected by filtration to a lesser extent. However, the nanobeads were subject to agglomeration and the observed intensity reduction was most probably related to settling, as
indicated by the observed increase of fluorescence intensity at the sediment surface (Fig. S3),
and to filter-feeding by the larvae, as indicated by the relatively higher reduction of intensity in
tanks with higher larval density (Fig. S2). We did not test photo-bleaching of the O₂ indicator
nanobeads (PtTFPP), but PtTFPP has a photo-stability similar to that of ruthenium and is less
susceptive to photo-bleaching than platinum octaethylporphyrin (PtOEP) (Yeh et al. 2006,
Borisov and Klimant 2009, Papkovsky and Dmitriev 2013). Nevertheless, the lifetime-based
measurement principle makes the τLIF O₂ imaging system independent of O₂ indicator
nanobead concentration and light distribution (Murniati et al. 2016) and independent of the
reduction in the intensity signal (Fig. S1).

The abundance of organisms used in our experiments (corresponding to 0–2448
larvae/m²) covered the range of C. plumosus densities frequently observed in ponds and lakes
around the globe (Granéli 1979, Hölker et al. 2015, Soster et al. 2015). Moreover, larval
behavior during the experiments and their activity patterns agreed with observations from
previous laboratory studies. Fine-scale O₂ measurements showed pumping activity in all
bioturbated tanks during the 1st measurement sequence, indicating that experimental handling
procedures did not greatly inhibit chironomid activity. By comparing different measurement
techniques, an average percentage of pumping activity of ~50% of the total observation period
also was estimated by Roskosch et al. (2011), with individual pumping sequences lasting, on
average, 2.75 min at 20–23°C and 100% O₂ saturation. Similar to our observations, pumping
activity increased from ~50% at O₂ saturation to nearly 100% under hypoxic (<20% saturation)
conditions (Walshe 1950). Besides burrow ventilation, the chironomids actively changed the
small-scale sediment topography by constructing chimneys at the burrow inlets. Our
observations clearly demonstrate that the up to 2-mm tall elongations of the burrow inlets
potentially tower over the DBL above the surrounding sediment surface and, thereby, bypass the low-O$_2$ concentration zone. Chimney construction has previously been described for *Chironomus riparius* larvae when exposed to hypoxic conditions (Stief et al. 2005).

Previous observations of burrow ventilation were focused mainly on the spatial and temporal dynamics of O$_2$ and other solutes within the sediment (Lewandowski and Hupfer 2005, Polerecky et al. 2006, Glud 2008, Baranov et al. 2016). Observations on the water side of the SWI have been restricted to pointwise concentration measurements with microelectrodes (Roskosch et al. 2011, Soster et al. 2015) or to observations of the flow fields associated with burrow ventilation using PIV measurements (Morad et al. 2010, Roskosch et al. 2010). Anoxic or suboxic plumes in the overlying water were documented in the presence of wide range of marine benthic organisms with the aid of planar optodes (Volkenborn et al. 2010, Woodin et al. 2010). However, these observations were limited to information next to rigid walls, which potentially affected plume dimensions and dynamics. The high-spatial resolution of the τLIF system applied in our study allowed us to quantify the dynamics of O$_2$ concentration gradients in the top few millimeters above the sediment surface. The observed spatial dimensions of the drawdown zones above burrow inlets and of the released plumes above the outlets principally agreed with measurements of the respective flow fields (Morad et al. 2010, Roskosch et al. 2010), but our measurements revealed the persistence of pumping-induced changes in the O$_2$ dynamics above the sediment–water interface. In the absence of bioturbation, O$_2$ transport into the sediment is limited by a DBL. The thickness of the DBL and, therefore the O$_2$ flux, is modulated by bottom boundary-layer turbulence driven by large-scale flows in the respective aquatic system (Lorke et al. 2003, 2012, Murniati et al. 2015). The DBL thickness and corresponding sediment–water fluxes of O$_2$ observed in the control tank without chironomids
were in the range of those values observed in a lake during periods of weak hydrodynamic forcing (Bryant et al. 2010). However, this relationship was altered completely by advective O₂ transport and the larger-scale mixing that was particularly generated by the energetic plumes released at the burrow outlet in the presence of chironomids. Our results demonstrated that even for the lowest organism density, O₂ transport became dominated by bioturbation that caused a complete change of the concentration boundary layer. Our experiments were conducted without significant background flow, but this finding suggests a regime shift from physical (large-scale flow fields driving bottom-boundary-layer turbulence) in the absence of bioturbation to biological (abundance and species composition of bioturbating fauna in the sediment) control of sediment O₂ uptake in the presence of bioturbating fauna. The biological control can result in strongly enhanced fluxes, but it is likely to have a different temperature-dependence (Roskosch et al. 2012, Baranov et al. 2016) than that of the hydrodynamic control or microbial O₂ consumption rate in the sediments (Murniati et al. 2015) and, therefore, can be expected to have different seasonal dynamics.

The effect of burrow irrigation on the O₂ dynamics above the sediment–water interface can be expected to depend on burrow depth and length, which we did not measure. The chosen sediment thickness of 5 cm may have been a limiting factor in our experiments. A wide range of the tube depths below the sediment surface has been observed. Granéli (1979) reported that the tubes can penetrate up to 15 cm into the sediment. Walshe (1950) found that the tubes usually extended to a depth of 3 to 6 cm, similar to what was reported by Soster et al. (2015). Nevertheless, in accordance with previous observations for similar larval density of 2000 individuals/m² (Polerecky et al. 2006, Soster et al. 2015, Baranov et al. 2016), the areal O₂ uptake rates of the sediment increased up to 2.5× in the presence of chironomids in comparison
to the diffusive flux in the control tank. Assuming a respiratory O$_2$ demand by the organisms at
the lower end of published data, the enhanced O$_2$ flux into the sediment exceeded the demand by
up to a factor of 4, which confirmed the potential importance of the prevailing controlling
mechanism (physical vs biological) for mineralization rates and therewith for nutrient and C
cycling in aquatic ecosystems (Granéli 1979, Hölker et al. 2015, Glud et al. 2016).

Given the potential significance of biological vs physical control of sediment–water
fluxes, future investigators should aim at a more realistic representation of both processes and
address mutual interactions of benthic boundary layer flow and bioirrigation. Such measurements
ideally would combine flow-field observations based on particle image velocimetry (Morad et al.
2010, Roskosch et al. 2010) with τLIF concentration imaging under defined background flow
conditions in laboratory or in-situ conditions without obstruction of the natural flow field.
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Author contributions: EM contributed to the experimental design, conducted the experiments, analyzed the data, and prepared the initial manuscript. DG provided his expertise in technical setup of the τLIF system. HH contributed to the detailed experimental design and together with EM analyzed the data under supervision of AL. AL proposed the idea and developed the concept of the experiment. KH prepared the O2-indicator-coated nanobeads and provided expertise related to the experimental design and data assessment presented in the manuscript. All authors reviewed the manuscript and gave final approval for publication.

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LITERATURE CITED


Lorke, A., B. Muller, M. Maerki, and A. Wuest. 2003. Breathing sediments: the control of diffusive transport across the sediment-water interface by periodic boundary-layer


FIGURE CAPTIONS

Fig. 1. A.—Photograph of the experimental setup showing the 4 test tanks in the temperature-controlled water bath and the lifetime-based laser induced fluorescence (τLIF) system consisting of laser and camera. B.—Illustration of the projected laser light sheet from above and the illuminated area captured by the camera. The interrogation area was 14.4 × 19.2 mm². C.—Typical burrow openings in the test tank. The image was taken in T1 before the start of the experiment. The dashed line shows the location of the light sheet on the selected burrow opening.

Fig. 2. The sediment–water interface (SWI) in the camera field of view showing the burrow inlet in T1 (A), burrow outlet in T2 (B), and burrow opening in T3 (C) at selected measurement times indicated in the legend. The vertical coordinate (z) corresponds to the distance from the SWI. The location of the burrow opening is indicated by the black bounding box. The white spaces between the lines are the regions where the camera view of the sediment–water interface was blocked by elevated sediment between the light sheet and the camera.

Fig. 3. Sequences of O₂ concentration distribution above a burrow outlet in T2 with a 10-s time interval between selected images (A–C) and a burrow inlet in T1 with a 12-s time interval between selected images (D–F). Animated sequences of both concentration distributions are available as supplementary videos V1 and V2.

Fig. 4. Percentage of time spent pumping vs O₂ concentration in the 3 test tanks. The dashed/dotted line shows a linear regression. O₂ saturation corresponded to a concentration of 280 µmol/L.

Fig. 5. Mean vertical O₂ concentration profiles in T0 (A), T1 (B), T2 (C), and T3 (D) at selected
measurement times indicated in the legend. The vertical coordinate \((z)\) corresponds to the
distance from the sediment–water interface, and the profiles were averaged laterally over
the entire field of view and temporally over the entire measurement sequence (10 min).

Fig. 6. A.—Time series of the height of the diffusive boundary layer \((\delta_{DBL})\) and the
corresponding \(O_2\) flux across the sediment–water interface (SWI) in T0. B.—Normalized
(nondimensional) mean \(O_2\) profiles in T0 for selected measurement times (cf. Fig. 5A).
The vertical coordinate \((z)\) was normalized using \(\delta_{DBL}\), whereas the \(O_2\) concentration \((C)\)
was normalized using the difference between the bulk concentration \((C_\infty)\) and the
concentration at the SWI \((C_0)\).

Fig. 7. Time series of mean \(O_2\) concentration \((C)\) in the 4 experimental tanks and the decrease of
the initial \(O_2\) concentration in T0 calculated from the estimated diffusive boundary layer
\((DBL)\) fluxes (Eq. 2).
Table 1. Experimental conditions in the 4 test tanks (T0–T3) including the starting and ending mean O₂ concentration during the measurement sequences, number and density of chironomid larvae at the start and at the end of the experiments, and number (n) of measurement sequences (10 min of O₂ concentration imaging each). The number in parentheses refers to the number of measurements sequences that passed the quality criteria and were used for analysis. Only the first 3 sequences were used for the mean vertical O₂ concentration profile analysis (bulk [O₂]).

<table>
<thead>
<tr>
<th>Test tank</th>
<th>Start</th>
<th>End</th>
<th>n</th>
<th>Bulk [O₂] (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>–</td>
<td>–</td>
<td>15 (10)</td>
<td>185–97</td>
</tr>
<tr>
<td>T1</td>
<td>3 (612)</td>
<td>2 (408)</td>
<td>15 (15)</td>
<td>191–154</td>
</tr>
<tr>
<td>T2</td>
<td>6 (1224)</td>
<td>6 (1224)</td>
<td>15 (15)</td>
<td>107–54</td>
</tr>
<tr>
<td>T3</td>
<td>12 (2448)</td>
<td>11 (2244)</td>
<td>15 (13*)</td>
<td>84–10</td>
</tr>
</tbody>
</table>
Table 2. Mean (±SD) values summarizing ventilation activity in the test tanks. The number in parentheses shows larval density (individuals/m²). After 4 h from the first measurement the O₂ concentration in T3 was <15 µmol/L. After 11 h from the first measurement, the pumping activity in T3 was no longer detected from the concentration image.

<table>
<thead>
<tr>
<th>Test tank</th>
<th>Pumping activity (min)</th>
<th>Individual pumping events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pumping</td>
<td>Resting</td>
</tr>
<tr>
<td>T1 (612)</td>
<td>5.1 ± 2.0</td>
<td>5.5 ± 2.1</td>
</tr>
<tr>
<td>T2 (1224)</td>
<td>6.5 ± 1.7</td>
<td>4.1 ± 1.7</td>
</tr>
<tr>
<td>T3 (2448)</td>
<td>8.3 ± 1.8</td>
<td>2.4 ± 2.2</td>
</tr>
</tbody>
</table>
Table 3. Components of the O₂ mass balance in the 4 experimental tanks during the first 6 h of the experiment. O₂ flux across the sediment–water interface (SWI) is the observed rate of change of O₂ concentration in the water \((\frac{dC}{dt} = \frac{V}{A} \frac{dC}{dt})\), where \(F_{\text{resp}}\) = areal O₂ flux corresponding to chironomid respiration (larval density \(\times\) individual respiration rate), the range corresponds to respiration rates between 2.9 \(\mu\)mol d\(^{-1}\) larva\(^{-1}\) (Granéli 1979) and 6.5 \(\mu\)mol d\(^{-1}\) larva\(^{-1}\) (Soster et al. 2015), \(\frac{F_{\text{dC/dt}}}{F_0}\) is flux enhancement factor relative to diffusive O₂ flux in T0 \((F_0)\), and \(\frac{F_{\text{dC/dt}} - F_0}{F_{\text{resp}}}\) is the ratio of the enhanced flux to chironomid respiration (the range of flux corresponds to the range of \(F_{\text{resp}}\)). The number in parentheses shows larval density (individuals/m\(^2\)).

<table>
<thead>
<tr>
<th>Test tank</th>
<th>(\frac{dC}{dt}) (mmol m(^{-2}) d(^{-1}))</th>
<th>(F_{\text{resp}}) (mmol m(^{-2}) d(^{-1}))</th>
<th>(\frac{F_{\text{dC/dt}}}{F_0})</th>
<th>(\frac{F_{\text{dC/dt}} - F_0}{F_{\text{resp}}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0 (0)</td>
<td>10.8</td>
<td>0</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>T1 (612)</td>
<td>10.1</td>
<td>1.8–4.0</td>
<td>0.94</td>
<td>–0.4 to –0.2</td>
</tr>
<tr>
<td>T2 (1224)</td>
<td>24.2</td>
<td>3.5–8.0</td>
<td>2.2</td>
<td>3.8–1.7</td>
</tr>
<tr>
<td>T3 (2448)</td>
<td>27.0</td>
<td>7.1–16</td>
<td>2.5</td>
<td>2.3–1.0</td>
</tr>
</tbody>
</table>