Magnetic Resonance Imaging as a Novel Method for Elucidating Sediment Burrow Structures and Functions

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ABSTRACT: Burrow structures produced by various benthic animals in sediments are important components of aquatic ecosystems, allowing the circulation of interstitial water via ingress of fresh bottom water into the burrows upon feeding and intraburrow migration. Although X-ray computed tomography has been used to visualize burrow structures, it could not reveal the structures in the soft mud in Lake Kasumigaura, where evaluation of the water-circulation effect of burrows is an important issue. Here, we describe the first attempt to use magnetic resonance (MR) imaging (MRI) to visualize intact burrow structures in the soft mud sediment cores collected from a eutrophic lake. Our MRI application clarified the dynamic distribution of burrows inhabited by chironomids in the soft mud that previous studies could not visualize. By examining the relationships between the degree of chloride ion depletion in deeper layers and the burrow density calculated from the MR images, we were able to consistently explain the water-circulation effect of burrows, suggesting the higher reliability of burrow density calculated from MR images. In addition, we were able to evaluate the activity of burrows, which is difficult to achieve in sediment core experiments. We observed a smaller water-circulation effect of burrows on ammonium ions than on chloride ions, suggesting the enhancement of ammonium production or release in burrow-rich sediments.

1. INTRODUCTION

Burrow structures in aquatic sediments are important ecological components, where benthic organisms inhabiting the burrows control various material fluxes across the water–sediment interface.1,2 Burrow structures are intimately involved in the circulation of bottom water with interstitial water via biological incorporation of fresh bottom water into them. This circulation function enhances the rate of phosphate liberation from sediments, increasing the eutrophication of lakes and ponds.3 Thus, there is an urgent need to study this important function (hereafter, “water-circulation effect of burrows”), especially in the organic-rich soft mud with a risk of hypoxia that generally prevails on the floor of eutrophic lakes, ponds, and estuaries.4,5 In the soft mud of freshwater bodies, burrows responsible for the water-circulation effect are 1–3 mm in diameter, and most of them are generated by chironomid larvae, which can occur at densities of more than 500 m−2.6 The quantification of burrow abundance is critical for estimating eutrophication risk.

Three-dimensional (3D) burrow structure analysis based on nondestructive visualization techniques such as X-ray computed tomography (CT) and magnetic resonance (MR) imaging (MRI) would be ideal for elucidating the relationship between the burrow abundance and the water-circulation effect of burrows. Although X-ray CT has been developed and used to analyze burrow structures in various types of sediments,6–13 it could not reveal burrow structures in the soft mud of Lake Kasumigaura, which has a high water content (i.e., 0.89 ± 0.03) and porosity (i.e., 0.95 ± 0.03). Therefore, we wondered whether MRI could be used for the analysis of the burrow structure.14 Here, we report on the novel use of MRI for burrow structure analysis in the soft mud of Lake Kasumigaura, one of the most eutrophic lakes in Japan (Figure S1).

Several studies evaluated the relationship between the burrow structures and the oxygen conditions and ion characteristics of interstitial water.15,16 In addition, the water-circulation effect of burrows was directly estimated by comparing water properties inside and outside the burrows, as measured by micrometer sensor probes17 or 2D planar sensors.17,18 Using X-ray CT, Michaud et al. revealed the 3D fine burrow distribution in sediments at a subtidal site.7 Dufour

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et al. used a similar method to visualize burrow structures in a high intertidal zone and analyzed the vertical spatial distribution of the burrows. These X-ray methods are effective only when there is a substantial difference in X-ray attenuation between inside and outside the burrow. However, we cannot easily discriminate the water-filled burrows in the organic-rich soft mud owing to the small differences in X-ray attenuation. In this study, we collected sediment cores from Lake Kasumigaura (water depth 6.2 m) and successfully scanned them using MRI both before and after incubation with chironomid larvae (*Chironomus plumosus*).

### 2. RESULTS AND DISCUSSION

MRI of control and chironomid-supplied cores revealed thick burrows elongating across the cores and many fine burrows in the upper half. This result suggests that the performance of MRI for analyzing the burrow structure is as good as that of X-ray CT, although the MRI resolution of 0.75 mm was poorer (i.e., generally 0.01 mm). Owing to the MR bias, MRI of burrow structures around the marginal side of the cores had two characteristics making their structures difficult to confirm. First, burrows at the upper and lower ends of the cores had weak signals. Irrespective of the burrow abundances in the cores, burrows in the top 1 cm layer were difficult to recognize, resulting in low burrow density (Figure 1a). A similar signal weakness should occur at the lower end of cores, although detecting this was difficult owing to the scarcity of burrows there. Second, as the magnetic force is directed from behind to the front of the cores (as shown in Figure 5b), there exist two vertical lines on the side curved plane of the cores where the magnetic lines of force vertically penetrate the side wall of the acrylic tube. Around the two vertical lines, burrow signals became weak and on the silicone sheet, these were discriminated as a thin line or a break (as shown at the arrow points in Figure 6c). To compensate for the site-specific MR bias, we could prepare calibration cores with many burrow mimics with the same water signal intensity distributed at the various sites of the cores, especially around the areas with strong MR bias. Another way to avoid this bias would be to exclude depths and sites under substantial MR bias. Owing to the difficulties in preparing ideal calibration cores, we adopted the latter method and compared layers at the same depth and excluded the top and bottom layers when we assessed the tube density across multiple depth layers (see Figures 3 and 4).

After incubation with chironomid larvae, many thick burrows clearly emerged across the cores (Figure 1a). The abundance of thick burrows during the incubation appeared to increase substantially in the chironomid-supplied cores because the burrow density calculated from the images significantly increased (Figure 1b), and also the probability of appearance of thick burrows increased (Figure 1c).

The number of thick burrows that can be counted by rotating 3D MRI should be consistent with the number of chironomid larvae creating U-shaped burrows one by one. The observed number of thick burrows in the control cores before incubation (i.e., 0 to 3 burrows per core) was close to the chironomid larval density estimated by monthly sampling with an Ekman–Birge grab in the field (mean ± SD, 0.56 ± 0.51 burrows per core, corresponding to 58.6 ± 53.6 m⁻², April 2013 to January 2015). In addition, the increase in the abundance of thick burrows after incubation (i.e., 7 to 10 burrows per core) was close to the number of larvae added during incubation (n = 9 per core). Therefore, we conclude that the thick burrows in MR images were the nests of the
incubated chironomid larvae. In contrast, the fine burrows were likely the nests of Naididae species (Figures 1a and S2). Although the number of fine Naididae burrows was much larger than that of thick chironomid tubes, their small contribution to burrow density had a limited effect on the effect of ion dilution. However, this finding does not indicate a small contribution of Naididae species in biogeochemical cycles within sediments.20 There were a few changes in burrow density during incubation in the control cores, but the degree of change was much smaller than that found in chironomid-supplied cores, suggesting that the effect of larval addition was more substantial than that of 2 weeks’ incubation alone.

Burrow structures that accelerate water circulation would rapidly eliminate ionic concentration differences between bottom water and interstitial water. To relate this water-circulation effect of burrows to the burrow density calculated from the MR images, we focused on the vertical profiles of chloride and ammonium ion concentrations in interstitial water. In Lake Kasumigaura, the deeper interstitial water has a higher chloride ion concentration because of a history of brackish water inflow (Figures 2a and S3).21 In deeper layers, (C1) were depleted relative to potential C0 (Figure S3). We would expect a much depleted ΔC1 (= C1 – potential C0) under a substantial water-circulation effect of burrows. By plotting ΔC1 against burrow density, we can describe the degree of dilution of ions by the following hyperbolic plot

\[ y = \frac{ab}{x + b} - a \]  

where \( x \) is burrow density, and \( y \) is ΔC1. The parameter \( a \) describes the y-value at the maximum x-value, and \( b \) expresses the sensitivity of the y-value to the increase of the x-value in the vicinity of \( x = 0 \). This nonlinear fitting function, called “Michaelis–Menten kinetics,” is used to express the catalytic ability of enzymes.22

By applying the different fitting curve of eq 1 to ΔC1 data at different depths, we can evaluate the sensitivity of the water-circulation effect to burrow density on the basis of \( b \) (i.e., for a smaller \( b \), a larger water-circulation effect is expected with a little increase in burrow density). The results of this fitting curve were significant only in the layers below 9 cm depth. The fitting was best in the deepest layer (Figure 3a), suggesting that the water-circulation effect of burrows could be easily found in the deeper layers. In addition, the smaller \( b \) value in the deeper layers indicates that the water-circulation effect per unit of burrow density was greater there. The circulation effect of burrows, known as biogenic advection, can be modeled by the cylindrical annulus of sediment structures, which creates coaxial-diluted zonation.23 In the deeper layers, the chloride ion concentration was much higher in interstitial water than in burrow water. This large difference would result in a larger diffusion flux from interstitial water to burrow water and an increase in the water-circulation effect per unit of burrow density (Figure 3a).

We also confirmed the circulation effect of burrows by analyzing the chloride ion concentration across the cores after 2-week incubation. The concentration in layers deeper than 6 cm of the chironomid-supplied cores was more depleted than that in control cores (Figures 3b and S3). This finding indicates that the water-circulation effect during incubation was greater in chironomid-supplied cores, owing to their higher burrow density.

MRI scanning allowed us to discriminate burrows filled with interstitial water, but not all the identified burrows were likely to have been active, such as abandoned burrows.23 With a high abundance of inactive burrows, no significant circulation effects by burrows would be expected, even in deeper layers. Therefore, we examined all data in the layers from 6 to 15 cm to discriminate whether there were layers rich in inactive burrows. Chloride ion depletion ratios, calculated as \(-\Delta C1/(\text{potential } C1 - C0)\), positively responded to \( \log(\text{burrow density}) \) in most of the chironomid-supplied cores (Figure 3c). In addition, some layers of the control cores did not respond well, suggesting that some layers in these cores were rich in inactive burrows, resulting in a poor relationship between the water-circulation effect and burrow density.

Water inside an active burrow tends to become aerobic. The ammonium ion concentration in interstitial water is decreased by two processes: direct dilution by the intrusion of bottom water with the low ammonium concentration into the burrow (Figure 2b) and ammonium consumption via nitrification near the inner wall of the burrow.2324 We recognized the dilution effect by burrows in layers deeper than 9 cm, suggesting that the former process functioned as in the case of the chloride ion.

![Figure 2](https://dx.doi.org/10.1021/acsomega.8b00192)
In addition, the smaller value in the deeper layers indicates that this dilution effect was dependent on the diffusion flux, which is proportional to the concentration difference between the pore and bottom waters. The nitrate plus nitrite ($\text{NO}_x$) concentrations in interstitial water increased as the ammonium concentration decreased (Figure 4b). Thus, nitrification contributed to the decrease in the ammonium concentration. Once ammonium is catalyzed to $\text{NO}_x$, these inorganic nitrogen forms can be removed from the interstitial water by gasification in denitrification and anammox processes.23−25 Considering that these two processes operated concurrently in the depletion of ammonium ions by burrows, we would expect a more profound dilution of ammonium ions than that of chloride ions. However, that is not what we observed. When the dilution of ammonium ions was plotted against that of chloride ions, the slope of the regression lines was <1, and the $y$-intercept was negative in both the chironomid-supplied and control cores (Figure 4c). In the chironomid-supplied cores, the dilution of ammonium ions was substantially smaller than that of chloride ions, suggesting that ammonium production is activated with the enhanced water circulation in thick chironomid burrows. Burrow host organisms often excrete diverse forms of labile organic nitrogen in their burrows. Aerobic conditions in the burrows accelerate the decomposition of the organic matter excreted from larvae.26 In addition, bio-irrigation enhanced ammonium fluxes from pore water to overlying water through burrows. Thus, increased ammonium production and flux through the burrows would be one reason for the lower apparent dilution of ammonium ions by burrows.

MRI allowed us to relate sediment burrow structures to the function of bioturbated sediments, even in the organic-rich fine-grained sediment of Lake Kasumigaura. The analysis of burrow structures in MR images made it possible to relate the extent of water circulation with burrow density and evaluate the degree of burrow activity in dilution and nitrogen mineralization. It is notable that burrow density was the most important parameter to predict the water-circulation effect at the interface between bottom water and interstitial water (Figures 3a and 4a). Aside from density, however, it is possible that burrow geometry such as tortuosity and the number of branches can change the ion concentration in interstitial water.26 By allowing the analysis of these complex burrow structures, MRI could perform advance research on diverse burrow structures and their functions. Thick chironomid burrows were active sites of ammonium production (Figure 4c), suggesting the link between burrow structures and biogeochemical functions in surface sediments.

### 3. CONCLUSIONS

3D MRI in control and chironomid-supplied cores before and after incubation revealed that the thick burrows were the nests of the incubated chironomid larvae. Burrow structures are intimately involved in the circulation of bottom water with interstitial water via biological incorporation of fresh bottom water into the burrows. MRI allowed us to relate sediment burrow structures to the water-circulation effect of burrows.
even in the organic-rich fine-grained sediment, where evaluation of the function of burrow structures was urgently needed. Burrow density estimated from MRI was the most important parameter to predict the water-circulation effect at the interface between bottom water and interstitial water.

4. MATERIALS AND METHODS

4.1. Preparation of Cores for MRI Scan. Six cores (i.d. 11 cm, length 50 cm) were taken at the center of Lake Kasumigaura, Ibaraki Prefecture, Japan (Figure S1), on March 4, 2014, by using an undisturbed sediment core sampler (HR
type, Rigo, Saitama, Japan). The water depth at the sampling point was about 6 m, and the sediment surface was usually oxic owing to the frequent mixing with the surface and bottom water and subsequent dissolved oxygen diffusion from bottom water to interstitial water. In the laboratory, the upper part (0–19 cm) of the sediment cores was transferred to the acrylic tubes of the same diameter that were equipped with bottom and top lids for MRI scanning (Figure 5a).

The sediment surface was set below 7.5 mm from the top of the acrylic tubes. The top lid has a hole (ø 5 mm) to release gas occasionally released from the cores and ensure oxic conditions of the sediment surface. We could not place the bottom lake water on the sediment surface because of the strong water signal on the MR image. Instead, we covered the sediment surface with a polyethylene film to prevent the surface from drying and maintain its high O2 permeability. The acrylic tubes for MRI scanning had seven holes (ø 2.6 mm) on the side that were usually sealed with Viton rubber (Figure 5a). The seals were peeled off when a portion of interstitial water was extracted. Before MRI measurement, the cores were kept in a 10 °C incubator. During MRI scanning, they were set up in the instrument under room temperature (ca. 25 °C) (Figure 5b).

4.2. Conditions for MRI Scanning. We conducted MRI measurements with a 4.7-T whole-body MR scanner for humans interfaced to an INOVA console (Agilent, Palo Alto, CA, USA) (Figure 5b). In preliminary experiments, we found that the interstitial water signal could not be detected in either gradient echo (GE) or spin echo (SE) images. A possible explanation is its magnetic susceptibility because of the heterogeneous nature of the sample, which causes short $T_2$ and $T_2^*$ of the interstitial water, meaning that the signal could not be detected at a short echo time (TE) of around 2 ms in the GE images and around 8 ms in the SE images. In contrast, chironomids nest in a burrow whose radius is much larger than the interstitial water. The nest burrow is filled with water, which we refer to as "burrow water." While the interstitial water could not be detected, burrow water could be measured by SE sequences with TE of around 8 ms. We measured $T_1$ and ($\approx 900$ ms) $T_2$ ($\approx 20$ ms) of burrow water.

We used a 3D SE imaging sequence with a matrix of 256 × 256 × 256 voxels for a field of view of 192 mm × 192 mm × 192 mm. Radio-frequency pulses with a width of 180 μs were used for excitation and refocusing to minimize the TE in order to decrease $T_2$ decay; TE was 8 ms, and repetition time (TR) was 800 ms. Optimized TR was measured by using the SE imaging sequence. As a result, the signal-to-noise ratio of burrow water was maximized at a TR of around 800 ms. The total scan time per core was 14 h 37 min. After scanning, a 3D image was obtained by reconstruction in ImageJ software.

4.3. Verification of MRI Detection Ability and MRI Data Screening. The 3D MRI data, obtained as image data of 256 × 256 × 256 voxels, had an actual spatial resolution of 0.75 mm × 0.75 mm × 0.75 mm. To verify the MRI detection ability, we checked the size and position of water-filled burrow structures by MRI (Figure 6a). Almost all portions of the tubes except the upper and lower sides were found at the indicated depth in the core (Figure 6a–c). To confirm that there was no significant skewness of the MRI data, we wrapped a 1 mm thick silicone sheet around the tubes. Because silicone produces good signals on images, we were able to discriminate the edge of the acrylic tubes as the white ring in each image (Figure 6c). The upper and lower sides of the white ring and the neighboring glass tubes were not well discriminated. In addition, MR signals were weak in deeper slices (e.g., 144- and 155-mm slices, as shown in Figure 6a).

On MRI, the burrows seemed to have smaller signals, especially at the upper and lower ends and marginal parts of
the cores (Figure 7). In particular, horizontal cross MRI had weak signals, especially on the marginal sides corresponding to the direction of magnetic force (i.e., upper and lower sides in the slice image of Figure 6c). As systematic signal weakness could be found in the fixed parts of the cores, most of this weakness should be attributable to the MR characteristic known as MR bias. Because the degree of signal weakness caused by MR bias was principally site dependent within a core, this effect should be similar in its strength between slices with the same depth in different cores. Thus, to avoid this bias, we excluded the data in the deepest layer under the MR bias (i.e., 15–18 cm) in the plots, including data across different depths (Figures 3c and 4c).

When screening MRI data, it was important to eliminate the nonburrow structures detected by MR. Nonburrow structures were not vertically continuous (e.g., see arrows in Figure 7). In cross-section, the diameter of nonburrow structures quickly varied, and the structures rapidly appeared and disappeared. In addition, both vertically and horizontally, nonburrow structures usually appeared in areas where the burrow density was low (Figure 7).

We screened MR images as follows. First, from the stacked longitudinal sections of the cores, we identified the depth where nonburrow structures began to appear. In most of the sediment core images, nonburrow structures can be easily discriminated by their splash-like appearance (see arrows in “before screening” images in Figure 7). However, when we found it difficult to classify a structure as burrow or nonburrow, we adopted the following criterion. In the cross-section at the depth of the structure center, we discriminated between structures by their spatial continuity in the 10 sections (i.e., 7.5 mm thickness) above and below the layer. Because burrow structures are well developed vertically relative to horizontally, this criterion resulted in the effective elimination of nonburrow structures (Figure 7). The ±7.5 mm thickness that we checked was sufficient to exclude the nonburrow structures, most of which extended vertically for less than 15 mm. Once we determined where nonburrow structures were aggregated, we checked whether burrow structures remained in those areas. Next, we deleted all nonburrow structures in the aggregated areas in ImageJ v. 1.50i online software (National Institutes of Health, Bethesda, MD, USA) to produce a screened cross-section for analysis. To screen the next cross-section, we referred to areas rich in nonburrow structures mentioned in the previous section. By confirming the boundaries of those areas in every section, we screened all cross-sections throughout the cores. Although our screening procedure could result in the minimum elimination of nonburrow structures, it is clear by comparing the longitudinal sections before and after screening that most of those structures were removed (Figure 7). In addition, cross-shaped white noise sometimes appearing around thick burrows with intense water signals was manually and appropriately handled by restricting the signal area to the area around the center of the burrow before burrow density analysis.

4.4. Experimental Conditions for the Incubation of Chironomid Larvae in Sediment Cores. An empty acrylic tube with the same diameter as the tube, as shown in Figure 5a, was adhered to the top of the acrylic tube for MRI scanning with a waterproof silicone sealant. Then, the upper tube was filled with lake water collected while we sampled chironomid larvae at the sampling site. The water sample in the upper tube to 3 cm above the sediment surface was treated as the bottom water sample. Nine chironomid larvae (9–12 mm in body length) were placed into each of three cores, and the other three cores were used as controls. All cores were incubated at 20 °C in a dark chamber for 2 weeks. Water in the upper tube was magnetically stirred gently during incubation in order to not resuspend the sediment surface.

4.5. Analysis of MRI Data for Burrow Areas, Burrow Density, and Probability Distribution of Burrow Size in Each Section. The MRI data of the cross-sections after screening were processed by using the particle analysis function in ImageJ (detailed at https://imagej.net/Particle_Analysis). After setting the image threshold intensity to 0.01–1.2, particle analysis of the 256 slices was performed to obtain...
the frequency distribution of the particle sizes in each slice. The threshold value was based on the ability to detect the water column within glass tubes, as shown in Figure 6. Burrow area in each slice was defined as the sum of particle sizes, and the burrow density was the ratio of burrow area divided by the cross-sectional area of the acrylic tube, that is, \( \pi (55 \text{ mm})^2 = 9503 \text{ mm}^2 \). To assess the relationships between interstitial water quality and these burrow parameters, we averaged the burrow parameters in each slice within the layers (i.e., 0–1.5, 1.5–3, 3–6, 6–9, 9–12, 12–15, and 15–18 cm). The burrow abundance and ion concentration data are tabulated in Table S1.

4.6. Interstitial Water Extraction and Water Property Measurement. Interstitial water samples were extracted at seven depths (i.e., 0.75, 2.25, 4.5, 7.5, 10.5, 13.5, and 16.5 cm) by inserting a Rhizon sampler (19.21.23, NL-6706, Daiki, Kounosu, Japan) 5 cm toward the center of each core via holes in the acrylic tubes (Figure 5a).

Between 1.0 and 1.5 mL of interstitial water was extracted. The probing area around the Rhizon sampler was within 3 mm of the skin of the sampler with a diameter of 2 mm. Thus, to be accurate, we considered the interstitial water extracted at a depth of D mm to be the interstitial water distributed from D − 4 mm to D + 4 mm. The concentrations of chloride, ammonium, nitrate, and nitrite ions were measured by ion chromatography (DX-320 with an eluent generator, EG40; Dionex, Tokyo, Japan). The detection limits ranged from 0.005 to 0.016 mg L⁻¹.

4.7. Evaluation of the High-End Concentration Profiles with the Least Burrow Water-Circulation Effect and Calculation of Relative Concentration Depletion. It is necessary to evaluate the degree of depletion of ion concentrations at depth \( z (C_z) \) relative to the cores with the least water-circulation effect in order to estimate the water-circulation effect of the burrows (see Figure S3). Ideally, we should take the ion concentration of cores with no burrows as the high-end concentration (potential \( C_z \)). However, because so few burrows could be found in any cores before incubation, we selected cores with the minimum burrow density as those with potential \( C_z \). The vertical concentration profile of Cores 1 and 4, in which the burrow areas were usually <20 mm² throughout the cores (see Figure 1a), was used as potential \( C_z \) before incubation.

The potential \( C_z \) of chloride and ammonium ions was the average \( C_z \) in Cores 1 and 4 before incubation (see dotted lines in Figures 2a,b and S3)

The degree of ion concentration depletion was the change in the concentration relative to the high-end concentration: \( \Delta C_z = C_z − \text{potential } C_z \). The ion concentration itself drastically increased from the surface to deep layers. Thus, even with similar burrow densities, both \( C_z \) and \( \Delta C_z \) tended to be large in deeper layers where the potential \( C_z \) was high. Thus, for the interlayer comparison of the effect of burrows, the ion depletion ratio [i.e., \( \Delta C_z / (\text{potential } C_z - C_0) \)] was the best parameter in the context of the offset of vertically different ion concentrations before burrow existence. Note that \( C_0 \) used in this calculation is the ion concentration of the bottom water before incubation. Under the maximum water-circulation effect of burrows, the concentration approaches \( C_0 \), the degree of concentration depletion approaches \( C_0 − \text{potential } C_z \) and the depletion ratio approaches 1.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.8b00192.

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Notes

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