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The role of tubificid worms (*Limnodrilus hoffmeisteri*) in sediment resuspension: a microcosm study

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Abstract – Sediment resuspension is an important internal lake process in regulating nutrient cycling and ecosystem structure. Tubificid worms are widely and abundantly distributed in freshwater ecosystems and are able to alter the sediment characteristics. This study was conducted to verify the hypothesis that the alteration of sediments by tubificids may substantially influence the sediment resuspension process. Specifically, we investigated the influence of *Limnodrilus hoffmeisteri* (Tubificidae) on sediment resuspension using an apparatus designed to simulate the sediment resuspension process in Lake Taihu (China). We examined *L. hoffmeisteri* according to its density (30 000 ind.m^{-2}) in Lake Taihu and simulated the light (3.2 m.s^{-1}), moderate (5.1 m.s^{-1}) and strong (8.7 m.s^{-1}) wind processes present in Lake Taihu. Tubificids loosened the sediment through their feeding and defecation activities and increased the sediment water content. The appearance of tubificids increased the suspended solids (SS) in a 1.6 m water column under all three wind processes. During the sedimentation process, SS decreased rapidly in both the control and tubificid treatments. The total SS in the water column was significantly increased by tubificids and it changed significantly with time. In addition, the small size particles of the SS in the tubificid treatment were higher than that in the control. So, the appearance of tubificid worms (*L. hoffmeisteri*) enhanced sediment resuspension and raised the proportion of small size particles in SS.

Key words: Sediment resuspension / suspended solids / tubificid worm / benthic animal / Lake Taihu

Introduction

Sediment resuspension refers to the redistribution process of sediment particles that have settled on the bottom back into the overlying water column, a process that can occur repeatedly (Bloesch, 1995). The increase of particles in the water column causes light attenuation and decreases the water transparency (James *et al.*, 2004; Sorokina and Kulygin, 2013). Accompanying sediment resuspension are an enhanced exchange between the porewater and overlying water and the adsorption and desorption of resuspended particles, factors that influence phosphorus and nitrogen cycling across the sediment–water interface (You *et al.*, 2007b; de Vicente *et al.*, 2010; Kalnejais *et al.*, 2010; Couceiro *et al.*, 2013; Tammeorg *et al.*, 2013). Consequently, the plankton and ecological structure in aquatic ecosystems are also changed (Schallenberg and Burns, 2004; Song *et al.*, 2010; Kang *et al.*, 2013). In addition, the transportation of metals and toxic organic pollutants in aquatic ecosystems are profoundly impacted by resuspension (Yang *et al.*, 2008; Kalnejais *et al.*, 2010; Superville *et al.*, 2014). Sediment resuspension is thus an important process in both limnology and environmental science.

Sediment particles are resuspended when the bottom shear stress exceeds the critical shear stress of the sediment bed (Evans, 1994; Bloesch, 1995). The critical stress is usually referred to as sediment erodibility, which is dependent on the physical (*e.g.*, particle size, bulk density and water content), geochemical (*e.g.*, organic content, clay mineralogy and pH) and biological properties of the sediment bed and the interactions among multiple properties (Grabowski *et al.*, 2011). Benthic animals live in or on the sediment, and the presence, feeding and egestion

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*Corresponding author: `cxfan@niglas.ac.cn`; `laoshuldudu@163.com` of these organisms produce structures (burrows, networks and biofilms) and substances (feces/pseudofeces and clusters), all of which influence the biological properties of the sediment (Widdows and Brinsley, 2002; Grabowski *et al.*, 2011).

Some bivalves, snails, polychaete worms and ghost shrimp decrease the critical shear stress of the sediment and increase sediment resuspension through increased sediment surface roughness and loosened sediment, and the mass of resuspended sediment is significantly correlated with the densities of these animals (Widdows *et al.*, 2000, 2009; Sgro *et al.*, 2005; Orvain *et al.*, 2006; Amaro *et al.*, 2007). However, the bivalve *Mytilus edulis* caused the highest sediment resuspension at a density of 25% of the sediment surface covered by the bivalve, and sediment resuspension decreased at bivalve densities above this level (Widdows *et al.*, 2002). In addition, increased density of the brittlestar *Amphiura filiformis* decreased the suspended sediment load (Amaro *et al.*, 2007). The bivalves *Actinonaias ligamentina* and *Ptychobranchus fasciolaris* did not influence the amount or particle size of suspended sediment (Zimmerman and de Szalay, 2007). Different taxa have distinct effects on sediment resuspension; thus, changes in the biotic community alter sediment erodibility (Widdows and Brinsley, 2002). Most published studies focus on invertebrates of relatively large size, although smaller invertebrates such as oligochaetes are also thought to influence sediment resuspension due to their ubiquity and high densities (Grabowski *et al.*, 2011).

Tubificid worms are widely distributed in freshwater ecosystem and can accumulate in high densities in eutrophic waters (Matisoff *et al.*, 1985; Cai *et al.*, 2010). Tubificids are conveyor-belt feeders that ingest particles in deep sediments and egest them on the sediment surface in the form of pseudofeces (Kaster *et al.*, 1984; Dafoe *et al.*, 2011). The activities of these worms alter sediment stratification (Matisoff *et al.*, 1999; Nogaro *et al.*, 2007; Dafoe *et al.*, 2011), increase sediment water content and porosity (Fukuhara, 1987; Zhang, 2010), change the distribution of the sediment particle size (Ciutat *et al.*, 2006; Zhang, 2010; Dafoe *et al.*, 2011) and alter the oxygen and nutrient dynamics in the sediment and across the sediment–water interface (Matisoff *et al.*, 1985; Mermillod-Blondin *et al.*, 2005; Zhang *et al.*, 2010). Thus, we hypothesized that tubificids may influence the process of sediment resuspension. Lake Taihu is a shallow eutrophic lake located in the delta of the Yangtze River in China and has an area of 2338 km^2 and a mean depth of approximately 1.9 m (Qin, 2008). Taihu is frequently influenced by winds, which induce the resuspension of sediment (Hu *et al.*, 2006; You *et al.*, 2007a). *Limnodrilus hoffmeisteri* is the dominant tubificid worm in this lake, and it can achieve a density of up to 30 000 ind.m^{-2} (Cai *et al.*, 2010). In the present study, we aimed to investigate the influence of *L. hoffmeisteri* on sediment resuspension using an apparatus that was designed to simulate the sediment resuspension process in Lake Taihu.

Materials and methods

Field sampling

The sediment cores used for the experiment were collected from Meiliang Bay (31°30′31.1″N, 120°10′31.0″E) in northwestern Lake Taihu. The surface sediment is constituted of 13.7% clay (0.02–4 μm), 69.8% silt (4–63 μm) and 16.5% sand (63–1000 μm). A gravity core sampler (11 cm internal diameter, 50 cm long, Rigo, Japan) was used to sample sediment cores. The sediment cores were stopped with rubber stoppers at both ends. Lake water was also collected with plastic barrels at the same time. A Petersen grab was used to collect the surface sediment to obtain tubificid worms. All samples were transported to the laboratory after they were collected.

Microcosms

To obtain uniform microcosms, the top 20 cm of each sediment core was sectioned in the laboratory into five layers of 4 cm depth. The same sediment portions from different cores were pooled together and sieved using a 0.6 mm mesh to exclude macroinvertebrates and large particles. Then, a dough mixer was used to fully homogenize each sediment pool. The five sediment pools were transferred in their original sequences and depths into six Plexiglas tubes (11 cm internal diameter, 30 cm long). Lake water was then added to the sediment surface in each microcosm using intravenous needles. Six microcosms with 20 cm of sediment and 10 cm of water were thus established. These microcosms were put into a water tank and submerged in lake water. The microcosms were pre-incubated for 1 month, after which they were randomly divided into two groups with three replicates. One group was assigned as the control (C) treatment, and no worms were added to this group. For the other group, 285 *L. hoffmeisteri* were introduced into each microcosm (30 000 ind.m^{-2}), and this group was designated as the tubificid (T) treatment. The experimental density of tubificids was based on their density in Lake Taihu (Cai *et al.*, 2010). After the tubificids were introduced, all microcosms were incubated for 2 months to allow the worms to achieve a stable state in the sediment. During the incubation, the overlying water was replaced every 2 weeks. The water in the tank was maintained at $20 \pm 0.5\,^{\circ}\text{C}$ with a circulator bath (Stik, China). To supply sufficient oxygen to the sediment and the worms, a mini-aerator was placed in the water tank to maintain O_2 saturation in the overlying water.

Resuspension apparatus

In the study, a Y-shape apparatus was used to simulate the sediment resuspension induced by winds (Fig. 1). The sediment core with several centimeters of overlying water was extruded from a cylinder into the apparatus (from the bottom). The sediment–water

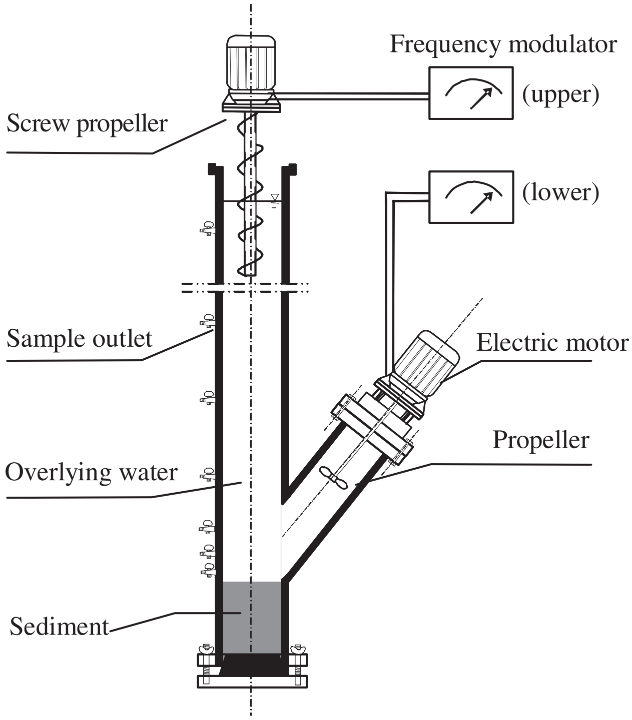

Fig. 1. Sketch of the Y-shape sediment resuspension apparatus.

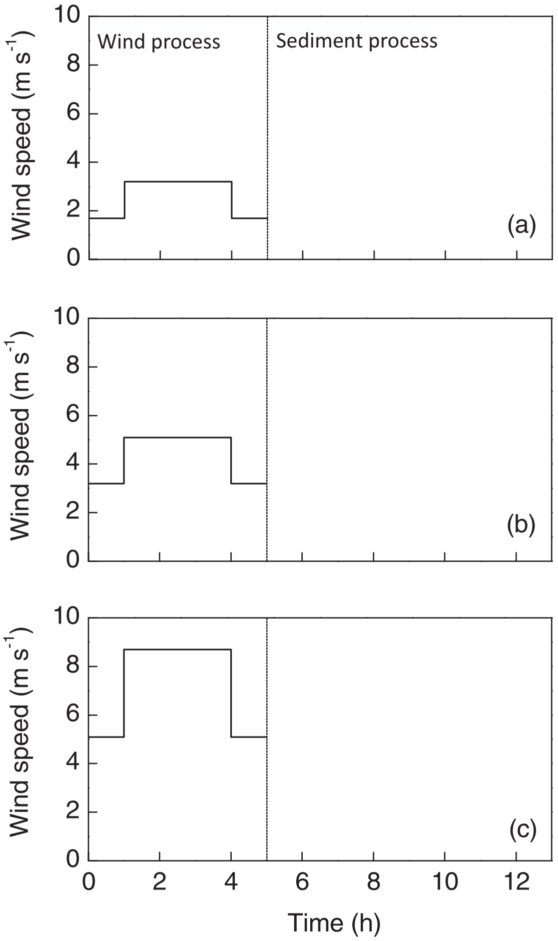

Fig. 2. Three simulated wind processes used to examine the influence of tubificid worms on sediment resuspension: light wind (a), moderate wind (b) and strong wind (c).

interface was kept horizontal to the turning point of the oblique tube as illustrated in the sketch in Figure 1. Then, the bottom of the apparatus was stoppered with a flange. Lake water was gently transported into the tube above the sediment to a depth of 160 cm. Shear stress was produced by the propeller on the electric motor of the oblique tube. The sediment particles were resuspended when the shear stress produced by the propeller exceeded the critical shear stress of the sediment bed. A screw propeller mounted inside the vertical tube 120 cm above the sediment–water interface was used to mix the water vertically. The rotating speeds of the two electric motors were separately controlled with a frequency modulator.

On the basis of the wind speed and the *in situ* observation of suspended solids (SS) in Lake Taihu (Hu *et al.*, 2006), You *et al.* (2007a) established the relationship between the rotation frequency (Hz) and wind speed (m.s^{-1}) through repeated simulations of sediment resuspension in the Y-shape apparatus. Four wind conditions – background, light, moderate and strong – were established on the basis of the wind speed and frequency at Taihu, with corresponding wind speeds of 1.7, 3.2, 5.1 and 8.7 m.s^{-1}, respectively. Corresponding to the four wind conditions, the respective rotation frequencies of the right motor were 5.8, 6.4, 7.1 and 8.4 Hz. In the present study, we simulated three wind processes, light, moderate and strong wind processes. For each wind process, it was constituted of 1 h starting wind, 3 h experiment wind and 1 h ending wind. The background, light and moderate winds served as the starting and ending winds for the light, moderate and strong wind processes separately. Take the light wind process for example, the background (starting) wind continued for 1 h, then wind was raised to the light wind speed and lasted for 3 h, then it was decreased to the background (ending) wind for 1 h (Fig. 2). The mean wind duration is about 3 h in Lake Taihu (You *et al.*, 2007b); this is why we selected 3 h as the lasting time of experiment wind in the study. After each wind process, the sedimentation process was observed for the subsequent 8 h. For details on the Y-shape apparatus and wind processes, see You *et al.* (2007a).

Resuspension experiment

Two months after the tubificids were added to the microcosms, the sediment surfaces of the T treatment were disturbed slightly with a hand-held propeller to obtain a smooth surface because the worms had created a rough surface through their activities and excretions. The heights of the sediment cores were measured, and the effect of the worms on the sediment height was examined. Then, all sediment cores were extruded into the Y-shape apparatus and lake water was added. An oxygen aerator was positioned 5 cm above the sediment–water interface to provide oxygen for the sediment and worms in

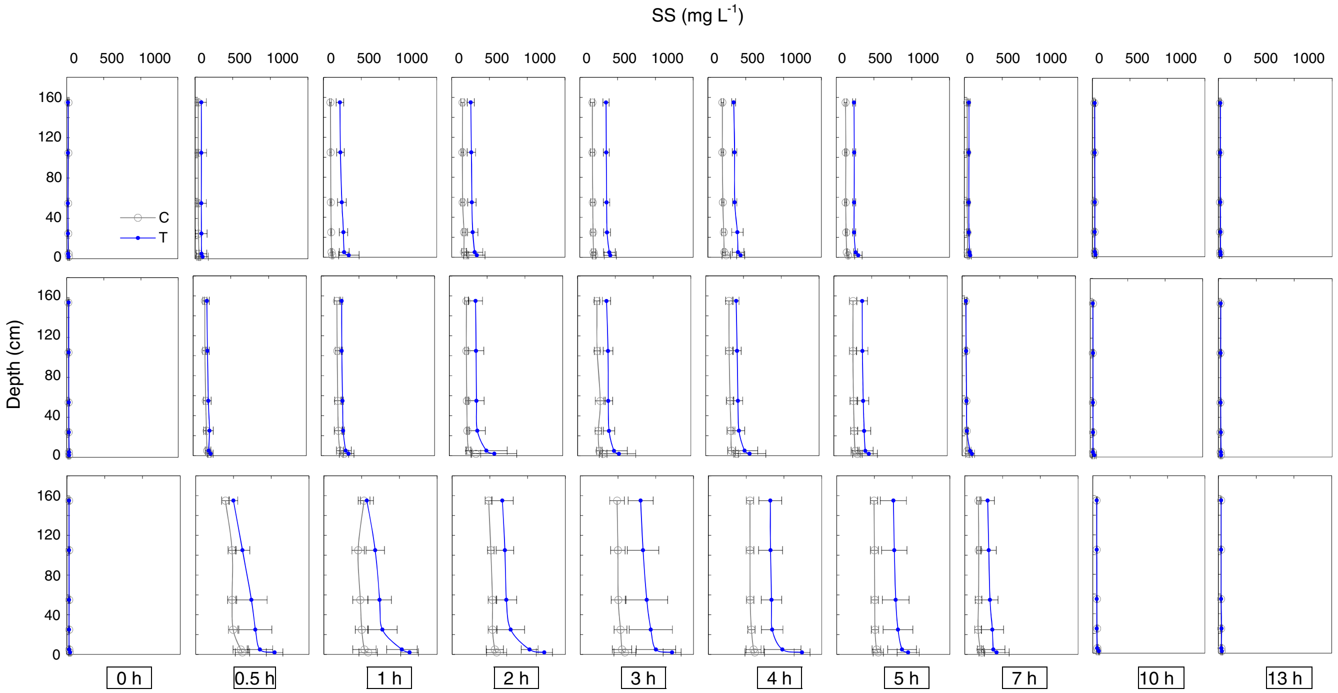

Fig. 3. Vertical distribution of suspended solids (SS) in the water column in response to three wind processes: light wind (upper panel), moderate wind (middle panel) and strong wind (lower panel). C = control treatment, T = tubificid worms treatment. Results are expressed as the means ± SD ($n = 3$).

each apparatus. The aerators were removed temporarily during the resuspension experiments. Over the next 3 days, the light, moderate and strong wind processes were simulated in sequence. During the wind and sedimentation processes, the SS in the overlying water was examined directly with an SS detector (Partech 740, UK) at 2, 5, 25, 55, 105 and 155 cm above the sediment–water interface. The room temperature was maintained at 20 ± 2 °C during the resuspension experiment.

At the fourth hour of the strong wind process, 500 mL of overlying water containing SS was sampled from each water column for particle size analysis. After the resuspension experiment, the sediment cores were unloaded and 0–0.5 cm of the surface sediment was collected and dried at 105 °C to a constant dry weight. The sediment water content (W, %) was calculated on the basis of the difference between the wet and dry weights of the sediment. The SS particle size distribution in the overlying water was examined with a laser size analyser (Malvern Mastersizer 2000, UK). The particle size parameters, $d(0.5)$ and $d(0.9)$, representing the mesh diameters through which 50 and 90% of the particles passed, respectively, were also calculated.

Calculation and statistical analysis

The total suspended solids (TSS, $g.m^{-2}$) in the water column were calculated as follows:

$$\mathrm{TSS} = \sum_{i=1}^{n} \mathrm{SS}_i \times \Delta h_i \qquad (1)$$

where SS_i is the concentration of SS in water layer i ($g.m^{-3}$) and Δh_i is the depth of layer i in the water column (m).

For TSS under each wind process, the difference between the C and T treatments was tested with a two-way analysis of variance (ANOVA) with treatment and time as two factors. The differences in water content and particle size parameter ($d(0.5)$ and $d(0.9)$) between treatments C and T were analyzed with a one-way ANOVA. The statistical analysis was performed using the SPSS software package (SPSS 13.0, USA).

Results

The activities of tubificid worms changed the sediment structure. Many tiny holes inhabited by worms could be seen along the Plexiglas tubes. The worms also excreted fecal pellets onto the sediment surface. After 2 months, the height of the sediment cores inhabited by worms increased by 1.27 ± 0.07 cm ($n = 3$) compared with the control treatment cores.

The wind processes resulted in similar resuspension and sedimentation processes for both the C and T treatments (Fig. 3). In the wind processes, the SS in the water column increased with time and reached its highest density at the third or fourth hour. The SS then decreased slightly with a decreased wind speed. When the wind processes ended (at the fifth hour), the SS decreased quickly over the subsequent 2 h and then gradually reached the level prior to the wind process during the last 6 h (7th–13th hour). The SS concentrations at different sites were similar in the upper water column, and the SS concentrations in the lower layers were higher than those in the upper layers. The light wind resulted in the lowest SS concentration in the water column, the strong wind resulted in the highest SS, and the SS in the moderate process was between the results of the light and strong winds. Compared with the C treatment, the worms created higher SS concentrations in the T treatments in response to all wind processes.

The calculated results indicated that tubificids significantly increased the TSS in the overlying water in the wind processes (Fig. 4 and Table 1). For example, TSS values were increased by 82.7, 33.8, and 51.7% at the fourth hour in light, moderate and strong wind processes, respectively. During the sedimentation process, the TSS in both treatments decreased quickly and became similar. The resuspension and sedimentation processes caused a significant change in SS over time (Fig. 4 and Table 1).

Tubificids changed the particle size distribution of SS in the water column (Fig. 5(a)). Specifically, the worms caused a decrease in the SS diameter compared with the C treatment, as indicated by the significant differences in *d*(0.5) and *d*(0.9) between the C and T treatments (Fig. 5(b)). The water content in the T treatments increased by 11.2% compared with the C treatment (Fig. 6).

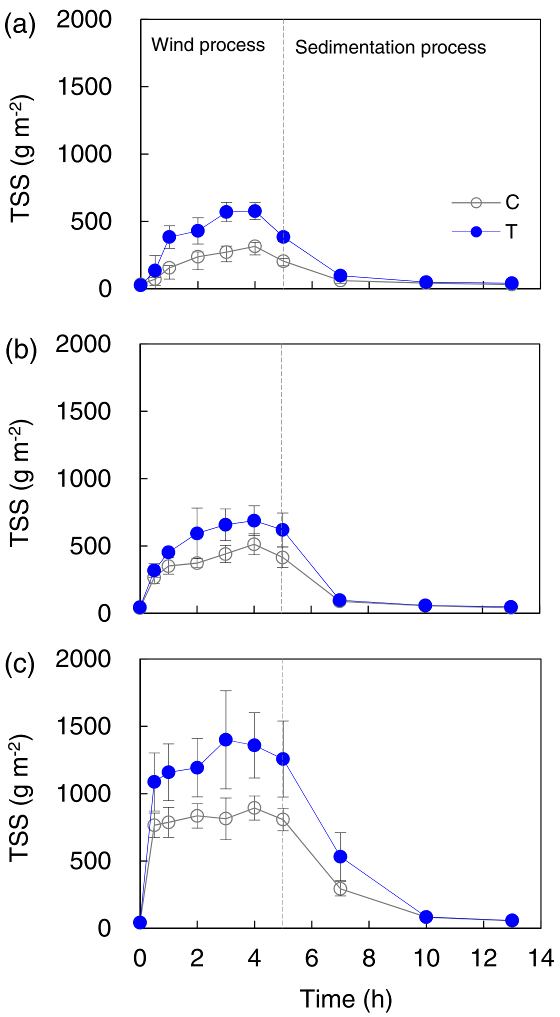

Fig. 4. Total suspended solids (TSS) in the water column in response to three wind processes: light wind (a), moderate wind (b) and strong wind (c). C = control treatment, T = tubificid worms treatment. Results are expressed as the means ± SD ($n = 3$).

Discussion

Sediment supplies living place for benthic animals, on the other hand, benthic animals alter the sediment structure and the biogeochemical cycling in sediment or across the sediment–water interface (Welsh, 2003; Nogaro *et al.*, 2006). As we know, sediment resuspension is also an important process influencing the biogeochemical cycling in aquatic ecosystem (You *et al.*, 2007a, 2007b; Kalnejais *et al.*, 2010). So, it is interesting to understand the role of benthic animals in sediment resuspension, which will give us more profound cognition in aquatic biogeochemistry. Some fishes and macroinvertebrates have been verified to influence sediment resuspension, and diverse results have been reported because they have different modifications on sediment erodibility (Flecker, 1996; Widdows *et al.*, 2002; Scheffer *et al.*, 2003; Orvain *et al.*, 2006; Statzner, 2012; Lin and Wu, 2013).

Tubificid worms ingest sediment particles at depth and defecate onto the sediment surface when they have burrowed into the sediment (Davis, 1974b; Kaster *et al.*, 1984). Their defecation rate is surprising; for example, the volume of sediment defecated by tubificids is 17 mL per worm per year at 10 °C (Davis, 1974a), and the defecated mass is 0.69 mg of fecal pellets per mg of dry worms per h (Kaster *et al.*, 1984). Owing to the worms' high defecation rate, a large quantity of fecal pellets cluster on the sediment surface. In addition, tubificids successively undulate their bodies to meet their respiration needs, which create tiny galleries in the sediment. Thus, tubificids loosen sediment consolidation (they increased the sediment height by 1.27 cm) and increase the water content (Fig. 6) and hydraulic conductivity of the sediment, in agreement with previous studies (Fukuhara, 1987; Nogaro *et al.*, 2006). The degree of consolidation is a key factor in determining sediment erodibility, and sediment with a higher water content is more likely to be eroded (Bale *et al.*, 2006; de Lucas Pardo *et al.*, 2013). The effect of tubificids on sediment water content and consolidation is thus a key reason for the increased sediment resuspension observed in our study. This result is in accordance with other studies investigating bivalves, snails, polychaetes and ghost shrimp (Widdows *et al.*, 2000, 2009; Orvain *et al.*, 2006; Amaro *et al.*, 2007).

Furthermore, the clusters of fecal pellets on the sediment surface are non-uniform, which results in an increased surface roughness. The rough sediment surface created by tubificids is also important for resuspension. Rough sediment is more easily resuspended than smooth sediment under the same conditions (Luettich *et al.*, 1990).

Table 1. Results of the two-way ANOVA for total suspended solids with treatment (control and tubificid worms) and time (0, 0.5, 1, 2, 3, 4, 5, 7, 10 and 13 h) as factors.

Wind	Factor	d.f.	*F*	*P*
Light	Treatment	1	104	< 0.001***
	Time	9	70.2	< 0.001***
	Treatment × time	9	8.84	< 0.001***
Moderate	Treatment	1	28.3	< 0.001***
	Time	9	60.0	< 0.001***
	Treatment × time	9	2.60	< 0.05*
Strong	Treatment	1	45.6	< 0.001***
	Time	9	51.6	< 0.001***
	Treatment × time	9	2.66	< 0.05*

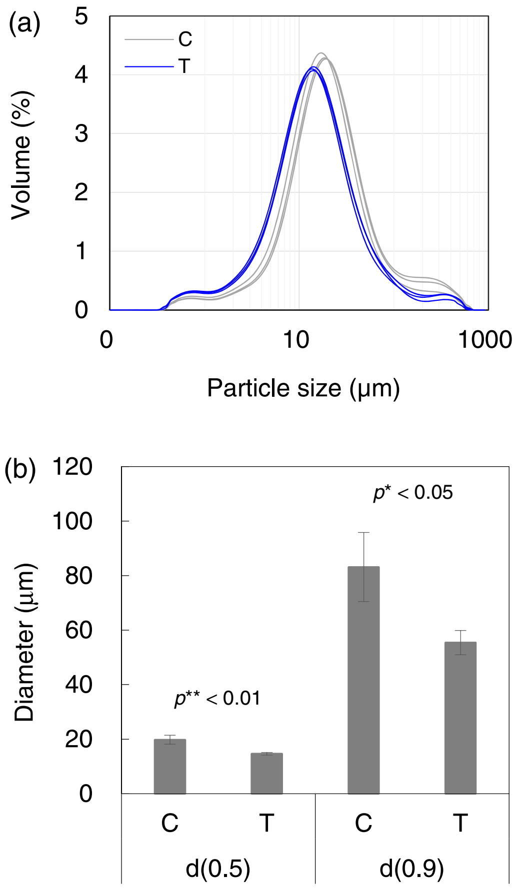

Fig. 5. Particle size distribution (a) and *d*(0.5) and *d*(0.9) of suspended solids from three control (C) and three tubificid worms (T) treatments (b). The *d*(0.5) and *d*(0.9) indicate that 50 and 90% of the particles were smaller than or equal to the respective diameter values, and results are expressed as the means ± SD ($n = 3$).

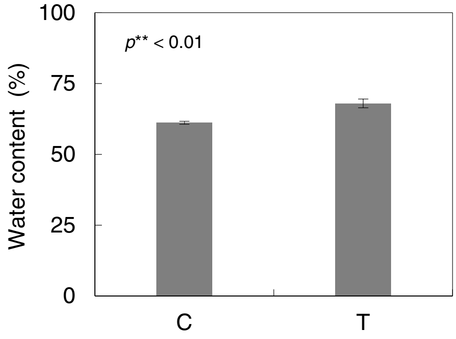

Fig. 6. Water content of the surface sediment (0–0.5 cm) from the control (C) and tubificid worms (T) treatments. Results are expressed as the means ± SD ($n = 3$).

Although we created a uniform sediment surface by rotating a propeller, successive defecation roughened the sediment surface again shortly after rotation or resuspension.

In both the control and tubificid treatments, more sediment was resuspended as the wind speed increased from light to moderate to strong (Fig. 4). The high wind speed corresponded to the higher rotating speed of the electric motor, which produced a greater shear stress. Our result is in accordance with previous reports that greater shear stress causes more particles to be resuspended (Sgro *et al.*, 2005; Bale *et al.*, 2006; Widdows *et al.*, 2009). It should be noted that more small particles were resuspended in the water column in the worm treatment compared with the control (Fig. 5). Tubificids increase the proportion of fine particles in the sediment surface by ingesting small particles in sediments and egesting them as fecal pellets at the sediment surface (Davis, 1974a; Rodriguez *et al.*, 2001; Ciutat *et al.*, 2006; Zhang, 2010). When resuspension occurs, the small particles defecated by the worms are resuspended into the water column.

The addition of benthic animals causes substantial destruction of the sediment surface, which differs from the natural state in that benthic animals act as an intrinsic part of the sediment. It was previously reported that unionid mussels (*A. ligamentina* and *P. fasciolaris*) decreased sediment stability in the first week after they were introduced into the sediment but increased sediment stability after 2 weeks (Zimmerman and de Szalay, 2007). The polychaete *Nereis diversicolor* did not influence the critical erosion velocity after 24 h of incubation (Widdows *et al.*, 2009); however, Fernandes *et al.* (2006) found that this species increased sediment stability (critical erosion velocity) after 20 days of incubation. A small time span (*e.g.*, 24 h) is not enough for sediment to achieve a realistic state after the introduction of benthic animals (Grabowski *et al.*, 2011). For this reason, the worms were incubated for 2 months in our study to allow them to arrive at a stable state.

Both sediment resuspension and bioturbation by benthic animals occur repeatedly in aquatic ecosystems. Thus, interactions between resuspension and bioturbation also occur repeatedly. The frequency of occurrence may be mainly determined by sediment resuspension, as bioturbation and the metabolism of benthic animals are more continuous than resuspension usually. This pattern is especially true for tubificids, because of their continuous ingestion, egestion, undulating movements and high densities in the sediment. The remarkable modification by tubificids on the sediment surface in still water after 2 months of incubation was not observed in the natural sediment in Lake Taihu, as this lake suffers frequent, typically daily, resuspension events (You *et al.*, 2007a). Therefore, before the microcosms were transferred into the Y-shape resuspension apparatus in the present study, the sediment surfaces of the tubificid treatments were disturbed slightly with a hand-held propeller to obtain a smooth surface. We believe this procedure enabled the resuspension simulation in the subsequent 3 days to more closely match the natural conditions in Lake Taihu.

Our results clearly indicate that tubificid worms enhance sediment resuspension. Sediment resuspension is an important process for oxygen and nutrient dynamics in aquatic ecosystems (Schallenberg and Burns, 2004; You *et al.*, 2007b; Tammeorg *et al.*, 2013). Tubificid worms and other benthic animals also significantly influence the oxygen and nutrient dynamics in the water, sediment and across the sediment–water interface (Lewandowski and Hupfer, 2005; Mermillod-Blondin *et al.*, 2005; Zhang *et al.*, 2010). For the importance of oxygen, nitrogen and phosphorus in global element cycling and eutrophication, the influence of the interactions between resuspension and benthic animals on oxygen and nutrient dynamics should be investigated in future studies.

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