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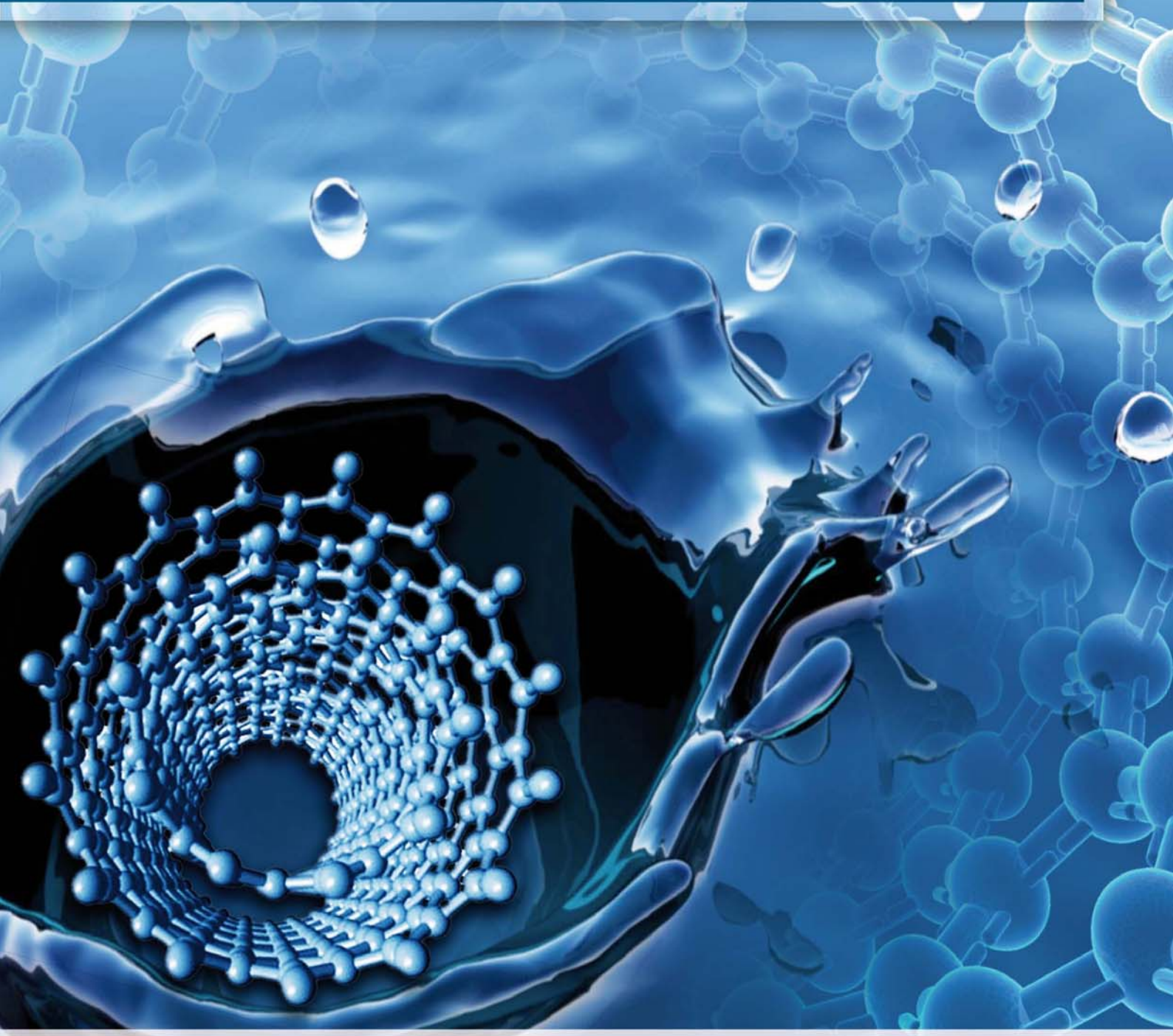

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# Systematic analysis of microfauna indicator values for treatment performance in a full-scale municipal wastewater treatment plant

Bo Hu, Rong Qi, Min Yang*

*State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China. E-mail: hubo106@163.com*

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**Abstract**

The indicator values of microfauna functional groups and species for treatment performance were systematically evaluated based on the continuous monitoring of the entire microfauna communities including both protozoa and metazoa over a period of 14 months, in two parallel full-scale municipal wastewater treatment systems in a plant in Beijing, China. A total of 57 species of ciliates, 14 species (units) of amoebae, 14 species (units) of flagellates and 4 classes of small metazoa were identified, with *Arcella hemisphaerica*, *Vorticella striata*, *Vorticella convallaria*, *Epistylis plicatilis* and small flagellates (e.g. *Bodo* spp.) as the dominant protozoa, and rotifers as the dominant metazoa. The abundance of the sessile ciliates was correlated with the removals of $BOD_5$ (Pearson's $r = 0.410$, $p < 0.05$) and $COD_{Cr}$ ($r = 0.397$, $p < 0.05$) while the testate amoebae was significantly positively related to nitrification ($r = 0.523$, $p < 0.01$). At the same time, some other associations were also identified: the abundances of the large flagellates ($r = 0.447$, $p < 0.01$), the metazoa ($r = 0.718$, $p < 0.01$) and species *Aspidisca sulcata* ($r = 0.337$, $p < 0.05$) were positively related to nitrification; the abundance of *Aspidisca costata* was correlated to the TN (total nitrogen) removal ($r = –0.374$, $p < 0.05$ ); the abundances of the sessile species *Carchesium polypinum* ($r = 0.458$, $p < 0.01$) and *E. plicatilis* ($r = 0.377$, $p < 0.05$) were correlated with the removal of suspended solids.

**Key words**: protozoan; metazoan; activated sludge; treatment performance; bioindicator

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## Introduction

Microfauna play important roles in municipal wastewater treatment systems. They maintain the density of bacteria, contribute to sludge flocculation and to some extent stimulate the bacterial activity in activated sludge systems, being responsible for the improvement of treatment systems (Curds, 1982; Pussard and Rouelle, 1986; Woombs and Laybourn-Parry, 1986; Martín-Cereceda et al., 1996; Ratsak et al., 1996; Pauli et al., 2001).

Microfauna have long been used as a bioindicator to evaluate the performance of biological wastewater treatment systems. In 1930s, Ardern and Lockett reported the association between some protozoan genera and the quality of treated effluents, which might be the first report on this subject (Curds and Cockburn, 1970). Based on the investigation of 56 sewage treatment plants in the UK, Curds and Cockburn (1970) reported that certain protozoan species were more frequently observed over certain effluent BOD (biochemical oxygen demand) ranges, which laid a solid base for relevant studies (Al-shahwani and Horan, 1991). Madoni (1994) synthesized previous findings, and proposed a "sludge biotic index" (SBI) for the evaluation of sewage treatment performance. Except for some special species like *Vorticella microstoma* and *Opercularia* spp., which are often related to bad conditions (e.g., lack of oxygen and occurrence of toxicants), sessile ciliates have been found to be the most important protozoa in activated sludge systems to indicate good treatment performance characterized mainly by high BOD removal, low effluent BOD and good effluent clarity (Madoni, 1994; Martín-Cereceda et al., 1996). At the same time, crawling ciliates have been found to be positively associated with BOD removal (Madoni, 1994), and testate amoebae have been considered as indicators for good nitrification performance (Madoni et al., 1993; Madoni, 1994), while swimming ciliates have exhibited tolerance to toxic and low dissolved oxygen conditions and their dominance has been associated with bad effluent quality (Martín-Cereceda et al., 1996).

To date, most of the relevant studies have mainly concentrated on the indicator values of ciliates (Madoni et

* Corresponding author. E-mail: yangmin@rcees.ac.cn

al., 1993; Martín-Cereceda et al., 1996; Lee et al., 2004), and to a lesser extent, testate amoebae (Madoni, 1994; Chen et al., 2004; Zhou et al., 2006). Although ciliates usually (and sometimes, testate amoebae as well) represent a dominant group of microfauna in activated sludge, there exist other groups including metazoa and flagellates, which should also play roles in the activated sludge processes. Moreover, microfauna compositions may be impacted by operational conditions (Salvadó et al., 1995), temperature (Martín-Cereceda et al., 2001), raw water quality, etc., and thus vary from one plant to another, which may lead to different relationships between the microfauna compositions and treatment performance (Curds and Cockburn, 1970; Salvadó et al., 1995; Chen et al., 2004). In order to better understand the indicator values of microfauna, knowledge of the dynamics of a wide spectrum of microfauna communities covering both protozoa and metazoa over a long period is therefore desirable.

In this study, the entire microfauna communities in two parallel full-scale municipal wastewater treatment systems in a plant located in Beijing, China, were continuously monitored over a period of 14 months. The primary objective of this study was to identify the indicator values of some special microfauna groups/species as well as to confirm the previously reported relationships between some important groups/species and system performance. The finding of this study could be helpful for achieving better operations of sewage treatment plants.

## 1 Materials and methods

### 1.1 Investigated systems

Two parallel full-scale municipal wastewater treatment systems in a plant located in Beijing, China, i.e., System 1 (anaerobic/anoxic/aerobic, $A^2O$) and System 2 (anoxic/anaerobic/aerobic, inverted $A^2O$), with treatment capacity of 200,000 $m^3$/day for each system, were investigated. The sludge recycling ratio was about 100% for both systems. The mixed liquor recirculation in System 1 was about 250%. The influent distributions to the anoxic and anaerobic tanks in System 2 were 30% and 70%, respectively. The flow diagrams of the two systems were outlined by Hu et al. (2012). A profile of the system parameters is shown in **Table 1**.

### 1.2 Sampling

Eighteen mixed liquor samples for microfauna analysis were collected from the end of the aeration tanks in each system from July 2009 to September 2010, with a sampling interval of 2 to 3 weeks for the most part. Samples were collected in a 2-L Plexiglas bucket and kept in suspension with a portable air pump until the completion of analysis. Samples from System 1 were taken 1 day after System 2 to assure that every sample could be analyzed in a timely fashion.

**Table 1** Parameters of Systems 1 and 2 during study period (July 2009 to September 2010)

| Variable | System 1 | System 2 |
|---|---|---|
| Influent $BOD_5$ (mg/L) | 193.2 ± 41.3 | |
| Influent $COD_{Cr}$ (mg/L) | 422.4 ± 88.0 | |
| Influent SS (mg/L) | 216.2 ± 66.3 | |
| Influent TN (mg/L) | 59.5 ± 7.8 | |
| Influent TP (mg/L) | 5.6 ± 1.0 | |
| Effluent $BOD_5$ (mg/L) | 4.9 ± 1.6 | 5.4± 2.2 |
| Effluent $COD_{Cr}$ (mg/L) | 49.0 ± 9.7 | 48.4 ± 9.5 |
| Effluent SS (mg/L) | 12.4 ± 2.3 | 11.9 ± 2.8 |
| Effluent TN (mg/L) | 16.8 ± 4.4 | 24.3 ± 6.6 |
| Effluent TP (mg/L) | 0.4 ± 0.5 | 0.3 ± 0.4 |
| Effluent $NO_3^-$ (mg/L) | 11.4 ± 4.6 | 10.3 ± 6.7 |
| $T$ (°C) | 21.1 ± 4.4 | 21.1 ± 4.4 |
| MLSS (mg/L) | 4093 ± 581 | 3670 ± 579 |
| SRT (day) | 14.2 ± 5.7 | 8.8 ± 3.5 |
| HRT (hr) | 13.6 ± 1.0 | 12.2 ± 1.0 |
| MLVSS/MLSS | 0.68 ± 0.08 | 0.67 ± 0.10 |
| SVI (mL/g) | 114 ± 52 | 141 ± 69 |
| DO (mg/L) | 1.60 ± 0.70 | 1.68 ± 1.30 |

$BOD_5$: five-day biochemical oxygen demand; $COD_{Cr}$: chemical oxygen demand with dichromate; SS: suspended solids; TN: total nitrogen; TP: total phosphorus; $T$: water temperature; MLSS: mixed liquor suspended solids; SRT: solids residence time; HRT: hydraulic retention time; MLVSS: mixed liquor volatile suspended solids; SVI: sludge volume index; DO: dissolved oxygen.

### 1.3 Microscopic analysis

Identification of protozoa was performed *in vivo* according to several keys (Kudo, 1966; Shen et al., 1990; Patterson, 1996; Foissner et al., 1999). Most protozoa were identified to the species level according to morphology and movements. Species not able to be identified to the species level were recorded as units, e.g., *Mayorella* spp. Small metazoa were classified into 4 units: rotifers, nematodes, gastrotrichs and tardigrades. Enumeration of protozoa and small metazoa was performed within 5 hr after sample collections (Madoni and Ghetti, 1981). Protozoa (except solitary small flagellates, e.g., *Bodo* spp.) and small metazoa in three replicates of 25 μL sub-samples were counted (Martín-Cereceda et al., 1996). Solitary small flagellates in five replicates of 0.1 μL sub-samples were counted using a Neubauer counting plate (counting chamber: 1 mm length × 1 mm width × 0.1 mm depth; XB-K-25, Shanghai Qiujing Biochemical Reagent & Instrument Co., Ltd., China) and recorded as one unit, i.e., solitary small flagellates. Species not detected in counting but observed in screening were recorded as 1 individual/mL (1 ind/mL) (Madoni, 1994).

### 1.4 Physico-chemical and operational parameters

Physical and chemical variables of the two systems were determined according to standard methods (APHA, 1995) on a daily basis and provided kindly by Beijing Drainage Group Co., Ltd., China (**Table 1**). Intraday values of these parameters were used in data analysis.

### 1.5 Statistical analysis

For illustration of the relationships between microfauna groups/species and system parameters, PCA (principal component analysis), Pearson correlation analysis and ANOVA (analysis of variance) were performed using STATISTICA Version 8.0. Removals of five-day biochemical oxygen demand ($BOD_5$), chemical oxygen demand with dichromate ($COD_{Cr}$), suspended solids (SS), total nitrogen (TN) and total phosphorus (TP) were calculated as the differences between the influent and the effluent: e.g., $BOD_{5\ removal} = BOD_{5\ influent} - BOD_{5\ effluent}$. Data (biotic and abiotic) for PCA and correlation analysis were logarithmically transformed, i.e., $x = \ln(x + 1)$, before analysis.

## 2 Results

### 2.1 Microfauna compositions

A total of 89 species and units of microfauna (protozoa and small metazoa) were observed in the two systems, including 57 species of ciliates, 14 species (units) of amoebae, 14 species (units) of flagellates and 4 units of small metazoa. Microfauna were further categorized into nine groups in terms of morphology, feeding habits and other lifestyles (Shen et al., 1990; Madoni, 1994; Martín-Cereceda et al., 1996; Liu et al., 2008), as shown in **Fig. 1**. The two systems exhibited similar microfauna compositions characterized with the dominance of small flagellates (SmF), sessile ciliates (SeC) and testate amoebae (TeA). The remaining 6 groups only contributed approximately 5% to the total population. As shown in **Table 2**, *Arcella hemisphaeri-*

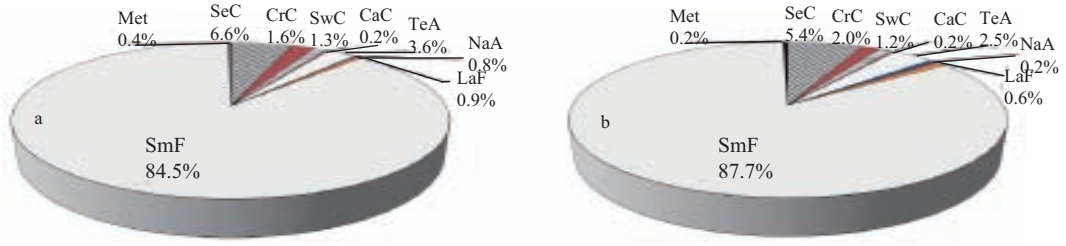


**Fig. 1** Average relative abundance of microfauna communities in Systems 1 (a) and 2 (b). SeC: sessile ciliates; CrC: crawling ciliates; SwC: swimming ciliates; CaC: carnivorous ciliates; TeA: testate amoebae; NaA: naked amoebae; LaF: large flagellates; SmF: small flagellates; Met: metazoa.

**Table 2** Frequency and abundance of microfauna groups and main species in Systems 1 and 2

| Groups/species | System 1 | | System 2 | |
|---|---|---|---|---|
| | Frequency (%) | Abundance (ind/mL) | Frequency (%) | Abundance (ind/mL) |
| **Sessile ciliates** | 100 | 5870 ± 3721 | 100 | 5871 ± 4291 |
| 1 *Carchesium polypinum* | 94 | 244 ± 264 | 89 | 148 ± 166 |
| 2 *Epistylis plicatilis* | 83 | 1491 ± 1588 | 89 | 764 ± 807 |
| 3 *Epistylis urceolata* | 94 | 283 ± 308 | 100 | 238 ± 229 |
| 4 *Vorticella convallaria* | 100 | 2096 ± 2177 | 100 | 1697± 2032 |
| 5 *Vorticella striata* | 100 | 1445 ± 1444 | 100 | 2560 ± 2499 |
| **Crawling ciliates** | 100 | 1385 ± 1356 | 100 | 2221 ± 2537 |
| 6 *Aspidisca costata* | 94 | 336 ± 368 | 100 | 1538 ± 2186 |
| 7 *Aspidisca sulcata* | 100 | 899 ± 1254 | 100 | 521 ± 547 |
| **Swimming ciliates** | 100 | 1140 ± 993 | 100 | 1320 ± 1401 |
| 8 *Trachelophyllum pusillum* | 94 | 959 ± 972 | 94 | 1223 ± 1359 |
| **Carnivorous ciliates** | 100 | 170 ± 163 | 100 | 206 ± 252 |
| **Testate amoebae** | 100 | 3192 ± 1835 | 100 | 2761 ± 3552 |
| 9 *Arcella hemisphaerica* | 100 | 2879 ± 1803 | 100 | 2572 ± 3412 |
| 10 *Euglypha tuberculata* | 100 | 272 ± 215 | 100 | 141 ± 150 |
| **Naked amoebae** | 100 | 724 ± 787 | 94 | 232 ± 297 |
| 11 *Mayorella* spp. | 100 | 722 ± 785 | 94 | 223 ± 289 |
| **Large flagellates** | 100 | 793 ± 534 | 100 | 602 ± 650 |
| 12 *Entosiphon sulcatum* | 39 | 180 ± 339 | 44 | 146 ± 328 |
| 13 *Peranema trichophorum* | 83 | 259 ± 285 | 78 | 130 ± 163 |
| 14 *Petalomonas steinii* | 33 | 140 ± 458 | 61 | 149 ± 526 |
| **Small flagellates** | 100 | 74646 ± 58769 | 100 | 95602 ± 75373 |
| 15 Solitary small flagellates | 100 | 73028 ± 57956 | 100 | 94556 ± 75349 |
| 16 *Sphaeroeca* sp. | 39 | 1618 ± 3205 | 50 | 1047 ± 1858 |
| **Metazoa** | 100 | 384 ± 404 | 100 | 249 ± 266 |
| 17 Rotifers | 100 | 301 ± 292 | 100 | 200 ± 192 |
| **Total microfauna** | – | 88305 ± 59596 | – | 109065 ± 75501 |

Ind: individual. Average abundance of the species listed in the table: > 100 ind/mL. –: no statistics.

*ca*, *Vorticella striata*, *Vorticella convallaria*, and *Epistylis plicatilis* were the dominant protozoan species, besides the most abundant solitary small flagellates, and rotifers were the dominant metazoa. The two systems were fed with the same raw wastewater and operated under similar conditions (**Table 1**). Therefore, the data acquired in Systems 1 and 2 were merged together in the following statistical analyses.

### 2.2 Associations between microfauna and performance parameters

PCA was performed to reveal the relationship between the microfauna groups/species and system parameters, as shown in **Fig. 2**, and the results of Pearson correlation analysis are shown in **Table 3**. Principal components derived from PCA are shown in **Tables S1** and **S2**.

As **Table 3** shows, 5 of the 9 microfauna groups (total

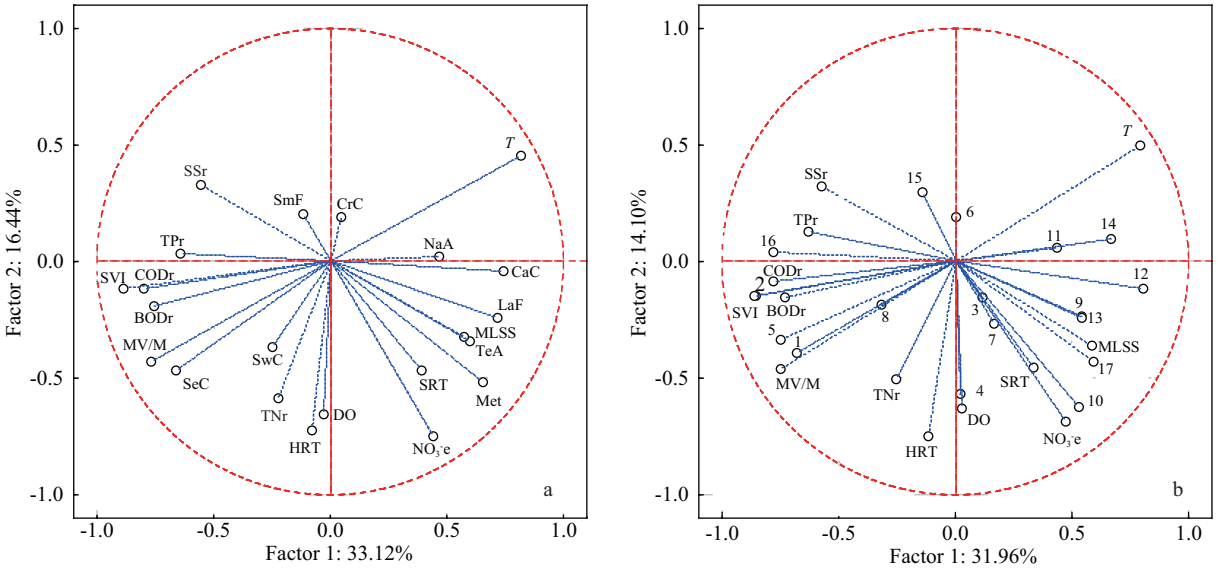


**Fig. 2** PCA analysis of microfauna groups (a) or species (b) and system parameters. SeC, CrC, SwC, CaC, TeA, NaA, LaF, SmF, Met: see **Fig. 1**. 1–17: species codes; species names see **Table 2**. BODr, CODr, SSr, TNr, TPr: removals of $BOD_5$, $COD_{Cr}$, SS, TN and TP, respectively; $NO_3^-$e: effluent $NO_3^-$; MV/M: MLVSS/MLSS.

**Table 3** Pearson correlation ($r$) between microfauna groups/species and performance parameters

| Groups/species | BODr | CODr | SSr | TNr | $NO_3^-$e | TPr |
|---|---|---|---|---|---|---|
| **Sessile ciliates** | 0.410* | 0.397* | 0.174 | 0.281 | –0.044 | 0.227 |
| 1 *Carchesium polypinum* | 0.448** | 0.472** | 0.458** | 0.183 | –0.152 | 0.186 |
| 2 *Epistylis plicatilis* | 0.609** | 0.642** | 0.377* | 0.284 | –0.406* | 0.436** |
| 3 *Epistylis urceolata* | –0.295 | –0.252 | 0.048 | –0.195 | –0.004 | –0.212 |
| 4 *Vorticella convallaria* | 0.033 | –0.055 | –0.197 | 0.003 | 0.142 | –0.159 |
| 5 *Vorticella striata* | 0.416* | 0.406* | 0.250 | 0.202 | –0.204 | 0.286 |
| **Crawling ciliates** | –0.141 | –0.119 | 0.013 | –0.243 | –0.006 | –0.317 |
| 6 *Aspidisca costata* | –0.123 | –0.091 | 0.107 | –0.374* | –0.189 | –0.284 |
| 7 *Aspidisca sulcata* | –0.057 | –0.079 | –0.198 | 0.097 | 0.337* | –0.313 |
| **Swimming ciliates** | 0.260 | 0.161 | –0.062 | 0.013 | 0.044 | 0.212 |
| 8 *Trachelophyllum pusillum* | 0.213 | 0.115 | –0.155 | –0.058 | –0.100 | 0.128 |
| **Carnivorous ciliates** | –0.503** | –0.589** | –0.576** | –0.046 | 0.289 | –0.530** |
| **Testate amoebae** | –0.364* | –0.389* | –0.394* | 0.008 | 0.523** | –0.319 |
| 9 *Arcella hemisphaerica* | –0.320 | –0.334* | –0.340* | –0.002 | 0.456** | –0.245 |
| 10 *Euglypha tuberculata* | –0.380* | –0.440** | –0.496** | 0.110 | 0.600** | –0.379* |
| **Naked amoebae** | –0.152 | –0.167 | –0.172 | 0.034 | 0.273 | –0.213 |
| 11 *Mayorella* spp. | –0.152 | –0.168 | –0.171 | 0.042 | 0.273 | –0.211 |
| **Large flagellates** | –0.340* | –0.447** | –0.449** | –0.117 | 0.447** | –0.335* |
| 12 *Entosiphon sulcatum* | –0.440** | –0.524** | –0.478** | –0.234 | 0.469** | –0.536** |
| 13 *Peranema trichophorum* | –0.133 | –0.245 | –0.403* | –0.083 | 0.364* | –0.340* |
| 14 *Petalomonas steinii* | –0.587** | –0.664** | –0.506** | –0.362* | 0.190 | –0.488** |
| **Small flagellates** | –0.064 | –0.033 | 0.150 | –0.061 | –0.234 | 0.301 |
| 15 Solitary small flagellates | –0.100 | –0.071 | 0.125 | –0.084 | –0.218 | 0.272 |
| 16 *Sphaeroeca* sp. | 0.509** | 0.586** | 0.480** | 0.317 | –0.354* | 0.472** |
| **Metazoa** | –0.379* | –0.429** | –0.423* | 0.115 | 0.718** | –0.283 |
| 17 Rotifers | –0.301 | –0.351* | –0.426** | 0.163 | 0.683** | –0.258 |

* $p < 0.05$, ** $p < 0.01$.

abundance) and 12 of the 17 main species (units) belonging to different groups exhibited significant correlations ($p < 0.05$) with the performance parameters.

The sessile ciliates, particularly the species *Carchesium polypinum*, *E. plicatilis* and *V. striata*, were positively correlated to the removals of $BOD_5$ and $COD_{Cr}$ ($p < 0.05$). The carnivorous ciliates, the testate amoebae, the large flagellates and the metazoa generally showed close (mostly significant) negative correlations with the removals of $BOD_5$, $COD_{Cr}$, SS and TP, while the testate amoebae, the large flagellates and the metazoa showed significant ($p < 0.01$) positive correlations with the effluent $NO_3^-$, which indicates the nitrification performance.

On the other hand, the species *C. polypinum* and *E. plicatilis* were positively correlated to the SS removal ($p < 0.05$). The *E. plicatilis* also showed significant correlations ($p < 0.05$) with the TP removal (positive correlation) and the effluent $NO_3^-$ (negative correlation). The species *Aspidisca costata* belonging to crawling ciliates exhibited negative correlation with the TN removal ($p < 0.05$) while the *Aspidisca sulcata* was positively related to the effluent $NO_3^-$ ($p < 0.05$). The *Sphaeroeca* sp., a colonial small flagellate, exhibited positive associations with the pollutant removals ($p < 0.01$, except for the TN removal) and negative association with the effluent $NO_3^-$ ($p < 0.05$) (**Table 3**).

## 3 Discussion

### 3.1 Microfauna community characteristics and system performance

According to previous studies, efficient activated sludge systems generally possess large amounts of microfauna with dominant sessile and crawling ciliates and very few flagellates: high abundance of ciliates ($> 10^7$ ind/L) usually indicates good BOD treatment and effluent clarity, while large number of small flagellates in mature systems are often associated with low DO (dissolved oxygen) and overloading conditions (Madoni, 1994). In this study, the total number of microfauna was about $10^8$ ind/L (**Table 2**) with a ciliate number close to $10^7$ ind/L ($8.6 \times 10^6$ and $9.6 \times 10^6$ ind/L on average in Systems 1 and 2, respectively), suggesting that the two systems were in general in good condition with respect to BOD and SS removals (average removal ratios of BOD and SS were 97.8% and 93.7% respectively in System 1, and the values were 97.2% and 94.9% in System 2). The existence of large numbers of small flagellates ($>10^7$ ind/L), however, suggested that the two systems may also be experiencing some problems. Abundant sessile ciliates and testate amoebae together with a large number of small flagellates could be described as the main features of the microfauna communities in the studied systems, which was different from the case observed several years ago, in which the crawling ciliates were more abundant than the testate amoebae and there existed very few small flagellates in the two systems (Liu et al., 2008). It was clear that the microfauna communities changed significantly over time. Overloading (each system accepted 225,000 $m^3$/day raw sewage on average in the most recent 3 years in comparison with the design capacity of 200,000 $m^3$/day) may be the main reason for this change in microfauna communities.

### 3.2 Indicator values of microfauna groups/species for system performance

As **Table 3** shows, 5 of the 9 microfauna groups (i.e., the sessile ciliates, the carnivorous ciliates, the testate amoebae, the large flagellates and the metazoa) and 12 of the 17 main species (units) exhibited good correlations ($p < 0.05$) with the treatment performance parameters in this study. Similar to previous studies (Madoni, 1994; Martín-Cereceda et al., 1996), the abundance of the sessile ciliates was closely correlated with the removals of $BOD_5$ (Pearson's $r = 0.410$, $p < 0.05$) and $COD_{Cr}$ ($r = 0.397$, $p < 0.05$), while that of testate amoebae was positively related to nitrification ($r = 0.523$, $p < 0.01$) (**Table 3**). However, the crawling ciliates did not exhibit significant correlations with treatment performance parameters, which was not in accordance with the previous study (Madoni, 1994). The species *A. costata* and *A. sulcata* belonging to crawling ciliates may have some potential to indicate the TN removal and the nitrification performance, respectively ($p < 0.05$).

On the other hand, the total abundance of the sessile ciliates was not significantly related to the removals of SS, TN and TP, suggesting that the indicator values of this group in terms of total abundance may only be restricted to BOD and COD removal performance. The sessile ciliates species *C. polypinum* and *E. plicatilis*, however, were positively ($p < 0.05$) associated with the removal of SS. The two species are believed to feed on suspended particles in the liquor phase (Fenchel, 1987; Madoni, 2011), and have been reported to be inversely related to effluent SS (Salvadó et al., 1995). As shown in **Table S3**, the two species have close correlations with the SVI (sludge volume index) values, suggesting that they may also be related with sludge bulking, which will be discussed in detail in a future publication focusing on sludge bulking.

The positive correlations between the abundances of the testate amoebae and the species *A. hemisphaerica* and *Euglypha tuberculata* and the effluent $NO_3^-$ ($p < 0.01$) was in accordance with previous studies (Madoni et al., 1993; Madoni, 1994), suggesting that this group may possibly be used as indicators for nitrification. As shown in **Fig. 2** and **Table S3**, testate amoebae were positively related to SRT (*E. tuberculata*: $r = 0.549$, $p < 0.01$) and MLSS (for total testate amoebae: $r = 0.516$; for *A. hemisphaerica*: $r = 0.452$; for *E. tuberculata*: $r = 0.587$; $p < 0.01$), and to some extent positively related to water temperature (for total testate amoebae: $r = 0.313$, $p = 0.0631$; for *A. hemisphaerica*: $r = 0.298$, $p = 0.0778$). It is known that

the testate amoebae have relatively low growth rates, especially under low water temperature conditions (Madoni, 1994), which could explain their correlations with SRT, MLSS and temperature, and possibly their indicator values for nitrification.

At the same time, some associations rarely reported by other researchers were also observed. Metazoa exhibited the highest correlation coefficients with the effluent $NO_3^-$ among all groups and main species ($r$ = 0.718 for the total metazoa and $r$ = 0.683 for the rotifers, $p < 0.01$), as shown in **Table 3**, suggesting that metazoa may be a good indicator for nitrification performance. Metazoa, particularly the rotifers, are believed to have a relatively long generation time (several days comparing with several hours for protozoa) (Poole, 1984) and a preference for higher DO (Sládeček, 1983) and temperature (Galkovskaja, 1987), which was demonstrated by the significant correlations in the present study between metazoa and some important variables for nitrification, such as SRT and DO ($r \geqslant 0.338$, $p < 0.05$; **Table S3**). Average abundances of the metazoa corresponding to the effluent $NO_3^-$ ($NO_3^-$e) ranges were: 119 ind/mL ($NO_3^-$e < 5 mg/L), 270 ind/mL (5 mg/L < $NO_3^-$e < 10 mg/L) and 601 ind/mL ($NO_3^-$e > 10 mg/L) (global ANOVA $p$ = 0.0009, **Table S4**). On the other hand, good nitrification could also be indicated by the total large flagellates ($r$ = 0.447, $p < 0.01$) and some large flagellate species (*Entosiphon sulcatum*: $r$ = 0.469, $p < 0.01$; *Peranema trichophorum*: $r$ = 0.364, $p < 0.05$). Average abundances of the large flagellates corresponding to the effluent $NO_3^-$ ranges were: 324 ind/mL ($NO_3^-$e < 5 mg/L), 629 ind/mL (5 mg/L < $NO_3^-$e < 10 mg/L) and 1215 ind/mL ($NO_3^-$e >10 mg/L) (global ANOVA $p$ = 0.0003, **Table S4**).

However, the testate amoebae (particularly *E. tuberculata*), the large flagellates (particularly *E. sulcatum* and *Petalomonas steinii*), the metazoa and the carnivorous ciliates, were inversely related ($p < 0.05$) to the removals of $BOD_5$, $COD_{Cr}$, SS, and in most cases, the removal of TP, as shown in **Table 3**. The abundance of testate amoebae species *A. hemisphaerica* was also found to be inversely related ($p < 0.05$) to the removal ratio of $BOD_5/COD_{Cr}$ by Chen et al. (2004). The carnivorous ciliates, the large flagellates and the metazoa were significantly ($p < 0.05$) positively related to water temperature, while the testate amoebae were partially related to water temperature (positive correlation, $p < 0.1$), as shown in **Table S3**. At the same time, the pollutant removals were inversely related to water temperature as shown in **Table S5** (for $BOD_5$, $COD_{Cr}$ and TP: $p < 0.05$), indicating that the systems removed more pollutants at low temperatures, perhaps due to the increase of influent $BOD_5/COD_{Cr}$/TP concentrations in winter and spring (data not shown). Further studies are required to clarify the indicator values of these four groups of microfauna.

Finally, the *Sphaeroeca* sp. belonging to small flagellates exhibited relatively strong correlations with the removals of $BOD_5$, $COD_{Cr}$, SS, and TP ($r$: 0.472–0.586, $p < 0.01$) and a negative correlation with the effluent $NO_3^-$ ($r$ = –0.354, $p < 0.05$) (**Table 3**, **Fig. 2**). However, this colonial small flagellate only appeared in a particular period from February to May, with a relatively low water temperature in the two systems. Karpov and Zhgarev (1981) reported that the species *Sphaeroeca volvox* was abundant in winter and spring in water reservoirs. Therefore, it seems that the *Sphaeroeca* sp. prefer low temperature. However, the indicator values of this species require further studies.

It is necessary to note that the relationship between the treatment performance and the indicator species could be impacted by different factors, such as the ranges of parameters (Salvadó et al., 1995), etc. Therefore, the indicator values of microfauna should be carefully evaluated, and simulated experiments are desirable to explore the relationship between the treatment performance and the indicator species.

## 4 Conclusions

The microfauna compositions and their correlation with the performance of two parallel systems in a full-scale municipal wastewater treatment plant were analyzed and the main conclusions are summarized as follows:

(1) In addition to the frequently studied ciliates and testate amoebae, large flagellates and metazoa were also found to be related with the treatment performance of the activated sludge systems, particularly for good nitrification performance.

(2) The sessile ciliates could be an indicator for good $BOD_5$ and $COD_{Cr}$ treatment performance. The species *Carchesium polypinum* and *Epistylis plicatilis* belonging to sessile ciliates could be indicators for good SS removal.

(3) The groups of carnivorous ciliates, testate amoebae, large flagellates and metazoa were found to be inversely related to the removals of $BOD_5$, $COD_{Cr}$ and SS. Their indicator values, however, need further confirmation.

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**Supporting materials**

Supplementary data associated with this article can be found in the online version.

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# Supporting materials

**Table S1** Principal components (factors) of system parameters and microfauna groups derived from PCA (the first 7 factors) (correlations ⩽ 0.25 were deleted)

| Variable | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 | Factor 7 |
|---|---|---|---|---|---|---|---|
| Sessile ciliates (SeC) | –0.664 | –0.467 | 0.342 | | | | |
| Crawling ciliates (CrC) | | | 0.719 | | | 0.477 | |
| Swimming ciliates (SwC) | | –0.368 | | –0.642 | 0.430 | | |
| Carnivorous ciliates (CaC) | 0.744 | | | | | | 0.252 |
| Testate amoebae (TeA) | 0.600 | –0.338 | | –0.279 | | 0.385 | |
| Naked amoebae (NaA) | 0.465 | | | | –0.281 | 0.651 | |
| Large flagellates (LaF) | 0.716 | | | –0.355 | | | |
| Small flagellates (SmF) | | | –0.429 | | 0.543 | 0.392 | 0.317 |
| Metazoa (Met) | 0.655 | –0.516 | | | | | |
| $BOD_5$ removal (BODr) | –0.755 | | –0.258 | | –0.404 | | |
| $COD_{Cr}$ removal (CODr) | –0.797 | | –0.252 | | –0.450 | | |
| SS removal (SSr) | –0.557 | 0.327 | | | | | |
| TN removal (TNr) | | –0.589 | –0.391 | 0.338 | –0.312 | | |
| Effluent $NO_3^-$ ($NO_3^-$e) | 0.443 | –0.749 | | | –0.261 | | 0.251 |
| TP removal (TPr) | –0.641 | | –0.581 | | | | |
| *T* | 0.817 | 0.457 | | | | | |
| MLSS | 0.575 | –0.319 | | | | | –0.676 |
| SRT | 0.391 | –0.464 | | 0.655 | | | |
| HRT | | –0.724 | | 0.419 | | 0.280 | |
| MLVSS/MLSS | –0.766 | –0.431 | | | | | |
| SVI | –0.884 | | | | 0.285 | | |
| DO | | –0.656 | 0.277 | –0.326 | –0.336 | | |
| Total variance (%) | 33.12 | 16.44 | 8.12 | 7.47 | 7.03 | 5.55 | 4.09 |
| Cumulative variance (%) | 33.12 | 49.56 | 57.68 | 65.15 | 72.18 | 77.73 | 81.82 |

*T*: water temperature; MLSS: mixed liquor suspended solids; SRT: solids residence time; HRT: hydraulic retention time; MLVSS: mixed liquor volatile suspended solids; SVI: sludge volume index; DO: dissolved oxygen.

**Table S2** Principal components (factors) of system parameters and microfauna species derived from PCA (the first 7 factors) (correlations ⩽ 0.25 were deleted)

| Variable | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 | Factor 7 |
|---|---|---|---|---|---|---|---|
| 1 *Carchesium polypinum* | –0.681 | –0.389 | | | –0.311 | | |
| 2 *Epistylis plicatilis* | –0.855 | | | | | | |
| 3 *Epistylis urceolata* | | | –0.541 | –0.323 | –0.446 | | |
| 4 *Vorticella convallaria* | | –0.568 | –0.511 | | | –0.515 | |
| 5 *Vorticella striata* | –0.751 | –0.336 | –0.354 | | | | |
| 6 *Aspidisca costata* | | | –0.891 | | | | |
| 7 *Aspidisca sulcata* | | –0.266 | –0.521 | | | 0.661 | |
| 8 *Trachelophyllum pusillum* | –0.314 | | | | 0.567 | | 0.511 |
| 9 *Arcella hemisphaerica* | 0.540 | | | | | | 0.502 |
| 10 *Euglypha tuberculata* | 0.533 | –0.620 | | –0.297 | | | |
| 11 *Mayorella* spp. | 0.439 | | | 0.467 | –0.447 | | 0.476 |
| 12 *Entosiphon sulcatum* | 0.805 | | | | | | |
| 13 *Peranema trichophorum* | 0.545 | | | 0.476 | | –0.416 | |
| 14 *Petalomonas steinii* | 0.665 | | –0.355 | | 0.279 | | |
| 15 Solitary small flagellates | | 0.297 | | –0.273 | | | 0.662 |
| 16 *Sphaeroeca* sp. | –0.780 | | | | | 0.343 | |
| 17 Rotifers | 0.590 | –0.432 | 0.440 | | | | |
| BODr | –0.729 | | | 0.532 | | | |
| CODr | –0.779 | | | 0.490 | | | |
| SSr | –0.573 | 0.320 | | | –0.385 | | |
| TNr | –0.255 | –0.506 | 0.493 | | | | |
| $NO_3^-$e | 0.473 | –0.686 | | | | 0.292 | |
| TPr | –0.629 | | 0.369 | | | | |
| *T* | 0.795 | 0.497 | | | | | |
| MLSS | 0.587 | –0.360 | | | | –0.327 | |
| SRT | 0.333 | –0.453 | 0.330 | –0.486 | –0.266 | | |
| HRT | | –0.751 | | –0.275 | –0.324 | | |
| MLVSS/MLSS | –0.747 | –0.462 | | | | | |
| SVI | –0.862 | | | | 0.259 | | |
| DO | | –0.628 | | 0.346 | | | –0.301 |
| Total variance (%) | 31.96 | 14.10 | 10.01 | 6.70 | 5.91 | 5.27 | 5.18 |
| Cumulative variance (%) | 31.96 | 46.06 | 56.07 | 62.77 | 68.68 | 73.95 | 79.13 |

**Table S3** Pearson correlation ($r$) between microfauna and operational parameters corresponding to PCA

| Groups/species | $T$ | MLSS | SRT | HRT | MLVSS/MLSS | SVI | DO |
|---|---|---|---|---|---|---|---|
| SeC | –0.847** | –0.067 | –0.069 | 0.446** | 0.764** | 0.680** | 0.267 |
| CrC | 0.079 | –0.014 | –0.241 | –0.108 | 0.003 | –0.035 | 0.103 |
| SwC | –0.465** | 0.005 | –0.215 | 0.115 | 0.294 | 0.430** | 0.222 |
| CaC | 0.542** | 0.316 | 0.314 | –0.020 | –0.470** | –0.569** | –0.068 |
| TeA | 0.313[a] | 0.516** | 0.197 | 0.195 | –0.384* | –0.390* | 0.114 |
| NaA | 0.424** | 0.244 | 0.014 | 0.006 | –0.384* | –0.458** | –0.112 |
| LaF | 0.467** | 0.599** | 0.296 | –0.063 | –0.555** | –0.567** | 0.228 |
| SmF | –0.022 | –0.178 | –0.065 | 0.029 | –0.015 | 0.219 | –0.297 |
| Met | 0.350* | 0.478** | 0.388* | 0.226 | –0.338* | –0.486** | 0.346* |
| 1 *Carchesium polypinum* | –0.689** | –0.173 | –0.002 | 0.484** | 0.619** | 0.609** | 0.119 |
| 2 *Epistylis plicatilis* | –0.747** | –0.361* | –0.121 | 0.265 | 0.704** | 0.704** | –0.078 |
| 3 *Epistylis urceolata* | 0.035 | 0.301 | 0.083 | 0.204 | 0.039 | –0.080 | 0.063 |
| 4 *Vorticella convallaria* | –0.311 | 0.418* | 0.043 | 0.298 | 0.181 | 0.164 | 0.301 |
| 5 *Vorticella striata* | –0.859** | –0.315 | –0.236 | 0.249 | 0.807** | 0.806** | 0.167 |
| 6 *Aspidisca costata* | 0.037 | –0.108 | –0.368* | –0.308 | –0.008 | 0.049 | 0.070 |
| 7 *Aspidisca sulcata* | 0.003 | 0.094 | 0.008 | 0.184 | 0.093 | –0.092 | 0.286 |
| 8 *Trachelophyllum pusillum* | –0.510* | –0.137 | –0.244 | 0.020 | 0.332* | 0.488* | 0.111 |
| 9 *Arcella hemisphaerica* | 0.298[b] | 0.452** | 0.126 | 0.162 | –0.371* | –0.339* | 0.073 |
| 10 *Euglypha tuberculata* | 0.078 | 0.587** | 0.549** | 0.375* | –0.117 | –0.324 | 0.288 |
| 11 *Mayorella* spp. | 0.419* | 0.254 | 0.020 | 0.014 | –0.374* | –0.453* | –0.113 |
| 12 *Entosiphon sulcatum* | 0.544** | 0.472** | 0.199 | –0.105 | –0.570** | –0.632** | 0.221 |
| 13 *Peranema trichophorum* | 0.289 | 0.483** | 0.095 | 0.009 | –0.349* | –0.403* | 0.170 |
| 14 *Petalomonas steinii* | 0.490** | 0.332* | 0.185 | –0.255 | –0.351* | –0.439** | 0.011 |
| 15 Solitary small flagellates | 0.006 | –0.155 | –0.055 | 0.022 | –0.041 | 0.184 | –0.291 |
| 16 *Sphaeroeca* sp. | –0.522** | –0.468** | –0.095 | 0.157 | 0.532** | 0.559** | –0.028 |
| 17 Rotifers | 0.269 | 0.436** | 0.353* | 0.251 | –0.287 | –0.433** | 0.338* |

* $p < 0.05$, ** $p < 0.01$. [a]$p = 0.0631$; [b]$p = 0.0778$.

**Table S4** ANOVA analysis of microfauna abundances over different $NO_3^-$e ranges

| Groups/species | Abundance (ind/mL) over $NO_3^-$e ranges | | | Global $p$ value |
|---|---|---|---|---|
| | 0–5 mg/L | 5–10 mg/L | > 10 mg/L | |
| Testate amoebae | 1578 ± 788 | 2948 ± 2010 | 4660 ± 4060 | 0.0217* |
| *Arcella hemisphaerica* | 1454 ± 744 | 2747 ± 1937 | 4205 ± 4014 | 0.0399* |
| *Euglypha tuberculata* | 106 ± 101 | 185 ± 91 | 348 ± 274 | 0.0054** |
| Large flagellates | 324 ± 214 | 629 ± 330 | 1215 ± 761 | 0.0003*** |
| Metazoa | 119 ± 128 | 270 ± 221 | 601 ± 445 | 0.0009*** |
| Rotifers | 112 ± 126 | 230 ± 210 | 439 ± 291 | 0.0029** |

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Ind: individual.

**Table S5** Pearson correlation ($r$) between wastewater treatment performance parameters and operational parameters derived from PCA

| Treatment performance | $T$ | MLSS | SRT | HRT | MLVSS/MLSS | SVI | DO |
|---|---|---|---|---|---|---|---|
| BODr | –0.618** | –0.341* | –0.339* | 0.069 | 0.492** | 0.606** | 0.210 |
| CODr | –0.598** | –0.447** | –0.341* | 0.056 | 0.494** | 0.599** | 0.187 |
| SSr | –0.164 | –0.389* | –0.252 | –0.059 | 0.179 | 0.375* | –0.137 |
| TNr | –0.296 | 0.053 | 0.324 | 0.463** | 0.346* | 0.071 | 0.207 |
| $NO_3^-$e | 0.059 | 0.310 | 0.386* | 0.435** | –0.035 | –0.328 | 0.659** |
| TPr | –0.380* | –0.239 | –0.282 | –0.053 | 0.408* | 0.513** | –0.146 |

* $p < 0.05$, ** $p < 0.01$.

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