Studies on the restoration succession of PFU microbial communities in a pilot-scale microcosm

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Abstract

In order to imitate the restoration succession process of natural water ecosystem, a laboratory microcosm system of constant-flow-restoration was designed and established. A eutrophication lake, Lake Donghu, was selected as the subject investigated. Six sampling stations were set on the lake, among which the water of station IV was natural clean water, and others were polluted with different degrees. Polyurethane foam unit microbial communities, which had colonized in the stations for a month, were collected from these stations and placed in their respective microcosms, using clean water of station IV to gradually replace the water of these microcosms. In this process, the healthy community in clean water continuously replaced the damaged communities in polluted water, the restoration succession of the damaged communities was characterized by weekly determination of several functional and structural community parameters, including species number (S), diversity index (DI), community pollution value (CPV), heterotrophy index (HI), and similarity coefficient. Cluster analysis based on similarity coefficient was used to compare the succession discrepancies of these microbial communities from different stations. The ecological succession of microbial communities during restoration was investigated by the variable patterns of these parameters, and based on which, the restoration standards of these polluted stations were suggested in an ecological sense. That was, while being restored, the water of station 0 (supereutrophication) should be substituted with natural clean water by 95%; station I (eutrophication), more than 90%; station II (eutrophication), more than 85%; station III (eutrophication), about 85%; station V (meso-eutrophication), less than 50%. The effects of the structural and functional parameters in monitoring and assessing ecological restoration are analyzed and compared.

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Keywords: Succession; Ecology restoration; Protozoa; PFU microbial community; Community parameter

1. Introduction

Succession in water communities is known to be influenced by environmental variables. Many works have been reported to study the structural and functional variables of aquatic communities in relation to the ecological succession (Kowalchuk et al., 2000; Kelly and Chistoserdov, 2001; Merila et al., 2002; Noll et al., 2005; Michail et al., 2005). Since the last decade of last century, nature management has embodied a lot of projects to restore damaged ecosystems (Hemerik and Brussaard, 2002; Clewell and Aronson, 2006). As a result, restoration ecology is becoming a scientific hotspot. Significant amounts of research have been conducted in an attempt to elucidate succession regularity of populations and communities during man-manipulated or natural restoration process of various ecosystems (Schrott et al., 2005). These ecosystems include grassland (Hemerik and Brussaard, 2002; Warren et al., 2002), bottomland hydrology (Kolka et al., 2000), shrubland (Calvo et al., 2002), semi-arid (Bonet, 2004), terrestrial ecology (Berg and Hemerik, 2004), forest ecology (Chen...
et al., 2003; Haeussler et al., 2004; Kauffman, 2004) and mine ecology (Grant and Loneragan, 2001). There are many methods to assess the ecosystem responses to environmental stresses, one of them is polyurethane foam unit (PFU) method. PFU microbial communities have been shown to form complex species assemblages, which exhibit many of the characteristics of structural communities (Xu et al., 2005). Various structural and functional characteristics of PUF microbial communities have been tested to be correlated with the degree of the lake’s eutrophication (Cairns and McCormick, 1993). The microbial communities are easier to be investigated and constitute important components of aquatic communities. Freshwater microbial is an interesting group, forming virtually self-contained communities that exhibit many characteristics of structure and function of entire aquatic ecosystems (Wang et al., 2004). The PFU method has been widely used to evaluate the environmental quality of natural waters in China and other countries (Xu et al., 2002; Morales-Morales et al., 2003; Ali et al., 2004; Wang et al., 2004).

Biomonitoring uses biological variables to survey the environment and is a complement to chemical monitoring. Assessment of ecosystem responses to environmental stresses is a new branch of biomonitoring (Wang et al., 2004). Protozoans play a key role in energy flow and mineral cycling in aquatic food webs. Because of their high species diversity, quick response to environmental changes and cosmopolitan distribution, protozoa have been increasingly recognized as good indicators of water quality, particularly with regard to organic pollution in lakes, rivers, reservoirs and oceans (Xu et al., 2005).

Lake ecological restoration is the process where water quality continuously improves and damaged communities gradually develop into healthy communities. Based on the process, we established a set of microcosm ecosystem, using natural clean water to lentamente replace waters with different polluted degrees to simulate the lake’s ecological restoration succession process. Although not directly implementable to a lake restoration, the experimental data could be theoretically useful to the real restoration of polluted water ecosystem. No studies of which we are aware have conducted such a microcosm restoration experiment to study the community parameters’ variables during restoration process. Furthermore, no studies have compared the natural succession of different impacted sites with that of a mature, relatively unimpacted site. The objectives of our study were to compare key community parameters across the successional gradient to determine the effect of succession and restoration on the water of these sites.

2. Materials and methods

2.1. Constant-flow-restoration system

In order to simulate the restoration process of natural water systems and ensure the facticity of ecological environment, we designed and set up a constant-flow-restoration system for the present research (Fig. 1). The system is composed of three parts: tanks, constant-flow regulation device, and test chambers (microcosms).

There are four tanks with total volume of 400 l, in which natural clean water was placed. Each tank is connected with tubes. Water in the tanks releases to a constant-flow regulation device, which has three layers and is made of acryl glass. The first layer is a flume into which clean water flows. Superfluous water is discharged in order to maintain a water height. The second layer is comparted into six small flumes. Water from the first layer is divided into the six small flumes by a flux governor valve, respectively. The valve can be moved vertically up and down to alter hydraulic pressures, its exit end can be put various types of medical needle head to modify the apertures. The water flux is in fact adjusted by both water press and apertures. Since the apertures of medical needle heads are less than 1 mm, the actual flux is the combined results that hydraulic pressure overcomes the capillary tension. The water flux \( Q \) is determined by the water altitude difference \( h \) and capillary aperture \( d \). When ignore trunking loss

\[
Q = \frac{\pi d^2}{4} \sqrt{2gh}
\]

where the accuracy of the flux governor valve is 0.5 ml/m.

There is an exit at the bottom of each small flume. Test chamber is the place in which the collected natural microbial communities are waiting for restoration lay. It is a cylinder made of acryl glass with diameter 40 cm and height 20 cm. Water from the small flume of constant-flow device flows via a hose tube into the microcosm. At the end of the hose tube connects a glass current divider, which is divided into six glass tubes (inner diameter 1 cm) equably disperse on the bottom of cylinder. This makes the water evenly mix
with the water in the microcosm. Water height in each microcosm is 12 cm and the capacity is 15 l.

2.2. Collection of microbial communities

Still continuing anthropogenic eutrophication of the Lake Donghu started in the 1960s when the phosphorus load began to increase. Since 1960s during the evolution of the lake’s state from oligotrophic to developed mesotrophic one, the structure of phytoplankton community dominating species was significantly changed as well as its total productivity. Six sampling stations were set up in the Lake Donghu (Fig. 2). Station 0 is located near the Shuiguohu Outlet, Station I is located in the middle of the Shuiguo Hu Bay, near the western end of the lake. Station II is in the central part of the Guozheng Hu area, Station III is in the central of Tanglin Hu, Station IV is located in the Hou Hu, and Station V is near the east end of Niuchao Hu. The stations represent different regions: the pelagic zone and a bay region. Stations 0, I, II, III, V and IV stand for, respectively, severely, heavily, moderately, slightly and unpolluted water (Jiang and Shen, 2003b, 2006; Jiang et al., 2007).

Microbial communities were collected by PFU method (Cairns et al., 1969). The microbial community mainly includes protozoa, fungi, algae and rotifers. In the present research, taxonomic parameters mainly aimed at protozoa. PFU may host most protozoans including planktonic, periphytic and benthic protozoan assemblages (Yongue and Cairns, 1973; Lugo et al., 1998). Non-taxonomic parameters dealt with all kinds of organisms in PFU. The PFU block was about 6.5 \times 6.5 \times 7.5 cm in size and blocks were soaked in distilled water for 24 h and squeezed before use. The PFU blocks were tied with thin ropes and placed at the depth of 1 m below the surface water.

2.3. Experiment set-up

Among sublakes of Lake Donghu, Houhu takes on the least wastewater. It is generally recognized that Houhu is unpolluted or just slightly polluted. So, in this study, water collected from station IV was put in the tanks as clean water to improve other stations. The experiment was conducted in winter and room temperature was 4–8°C, the water sampling from station IV was taken every other day to replace the old water in tanks. Water sample was not given any treatments to maintain its organisms a natural status.

Water and communities in stations 0, I, II, III, V were tested samples. Each 15 l of water from these stations and station IV was enclosed in test chambers, respectively, the chamber of station IV was controlled sample. Each station was set a parallel tested chamber. So, there were six tested groups and totally twelve tested chambers.
The tested communities were exposed to the species source pool from the Station IV, which provided the water source for the experiment. The restoration was, on one hand, the process of polluted water being replaced continuously by clean water, and on the other hand, the succession of healthy communities constantly mixing with damaged communities and leading the latter to continually develop into healthy communities.

Tested PFU communities were sampled from stations 0, I, II, III, IV, V PFU blocks were exposed in each station for a month and microbial organisms had formed mature PFU microbial communities and had reached equilibrium.

PFU samples were hanged on their respective chambers, each chamber was put five PFU blocks, evenly distribution. During the experiment, intensity of illumination was about 1000 l×(12 cm height form water surface) and Illumination periodic time was 16:8 D. Two rows of frame were covered with plastic membrane to decrease the inbreak of various spores. PFU microbial communities were domesticated for 24 h, and then each chamber began to take on clean water of station IV. The restoration experiment continued for five weeks, clean water flowed into chambers at the speed of 1 ml/m to restore the water and microbial communities of each tested stations. Water substitute rate at any time was calculated as

\[ T = \frac{V}{R} \ln(1 - P) \]  (2)

where \( V \) is the volume of chamber, here is 15 l; \( R \) the flow speed, here is 1 ml/m; \( T \) the experiment time and \( P \) the water substitution rate.

A PFU sample of each station was examined in the laboratory within five hours after collecting from each station. PFU samples in microcosms were examined every week. Sampling of the PFU from chambers should be at random, one block was taken out each time. For each station, there were two PFU blocks from two paralleled chambers that should be examined. When sampling, the string was gently taken off, and the PFU was lifted out of the test chamber. After the water was squeezed in a beaker, the PFU was gently placed back into the chamber and retied. A mark should be made to indicate that the PFU has been sampled and should not be sampled again. Three slides from each sample were examined sequentially under high, medium and low amplifications for species identification in order to observe more species.

3. Community parameters

3.1. Species number and percentage of Phytomastigophora

Protozoa species (including Phytomastigophora, zoo-mastigophora, sarcodina and ciliata) in the squeezed liquid from PFUs were observed and identified under the microscope for species number (\( S \)), percentage of Phytomastigophora. For microscopic determination, pipette is used to take three drops of solution from the bottom of the beaker to the slide. The first slide is observed under the high amplification of the microscope and the second and third slides are observed under the middle and low amplifications.

3.2. Diversity index

Gently shake the beaker, pipe 0.1 ml of water sample to a 0.1 ml counting grid and cover with cover glass for counting the number of living protozoans. Now there are species number (\( S \)) and abundance (\( N \)), diversity index was calculated by maglaef's (Margalef, 1969) formula

\[ D = S - 1/ \ln N \]  (3)

3.3. Similarity index

The relationships among the communities were estimated according to their phenetic similarities, this parameter was estimated by counting the percentage of common species between any two stocks, using the following formula:

\[ F = \frac{N_{xy}}{N_x + N_y} \]  (4)

where \( N_x \) and \( N_y \), respectively represent the total number of species recorded in stocks \( X \) and \( Y \), and \( N_{xy} \) represents the total number of common species recorded between communities \( X \) and \( Y \). Phenetic distances are related to phenetic similarities by \( D = 1 - F \). The unweighted pair group method of analysis (UPGMA) was used to examine similarities among stations based on PFU protozoa communities’ composition by the software NTSTSp.
3.5. Heterotrophy index

The calculation formula for the heterotrophy index is as follows:

\[ HI = \frac{B}{Chla} \]  

Biomass (B) can be expressed by ash-free dry weight. Chlorophyll a (Chla) and ash-free dry weight measurements were made from microbial communities collected from PFU according to the methods described by Vollenweider (1969), with modifications as described in Standard Methods (APHA, 1976). 10 and 20 ml of PFU extrusive liquid were used for the determination of ash-free dry weight and chlorophyll a, respectively. Higher HI means the water quality is poorer.

4. Results

4.1. Water substitution rate in each week

According to formula (2), when the flow rate of clean water was 1 ml/m, each week when the PFU communities were examined, the water substitution rate in chambers was calculated (Table 1). At the end of the fifth week, the replaced rate of tested water with clean water in each chamber was over 96%, the restoration was basically ready.

4.2. Species number

A total of 253 species of protozoa were observed, among which 73 were Phytomastigophora, 44 were zoomastigophora, 40 were sarcodina and 96 were ciliata. From week 0 to week 5, the total species were 133, 134, 135, 138, 105, 94, respectively showing degressive tendencies. Table 2 lists the taxa composition and total number of protozoan species collected from PFUs at the six stations during restoration.

The variables of the species number in each station during restoration is shown in Fig. 3. At week 0, species numbers of stations 0, I, II, III, IV, V were 54, 53, 39, 19, 46, 42, respectively. At the early stage of restoration, the variables of the species number of tested microbial communities increased gradually. At the third week, the species number of station 0 reached the maximum (66), and then began to decrease. Species numbers of stations I and II reached maximum (67, 52, respectively) at the second week. Afterwards, the species numbers of the two stations decreased as well. The situation of stations III was special, its species number increased all along from week 0 (19) to week 5 (39), whereas species number of station V had not an obvious variable during the restoration period, keeping in about 45, except the 55 at week 3. On the contrary, the species number of station IV was stable at the scope of 40–45 during this time.

4.3. Composition of Phytomastigophora

The total percentage of Phytomastigophora in microcosms is calculated from Table 2. The variable tendency of percentage of Phytomastigophora of tested communities was ascending during the period (Fig. 4). Although the increase in the percentage of Phytomastigophora was not distinct for each station for the whole system, it increased from the 24.62% of week 0 to 37.23% of week 5. This reflected that the autotrophic component in communities was increased during restoration (Fig. 5).

4.4. Diversity index

As a whole, maglaef diversity index of the stations increased gradually during restoration process (Fig. 6). Similar to the situation of species number, the fluctuation of DI of station V was the least and the DI of station III was increasing constantly during the restoration period. The variables of DI in stations 0, I, II was greater, the values reached the highest at third week, and then decreased. At the end of restoration, DI of each station was close to the value of station IV and the values were in a narrow scope of 3.81–3.91. From the present results, maglaef DI was effected greatly by species number, the larger the species number was the greater the DI would be. While the richness had a little influence on DI, because the logarithm of richness made the difference among samples decrease greatly.

4.5. Community pollution value (CPV)

CPV of each station was calculated from SPV (Jiang and Shen, 2003a, 2005; Jiang, 2006) and is shown in Fig. 7. According to chemical analysis, water qualities become worse in the order of stations IV, V, III, II, I, 0 in turn. CPVs of stations 0 to V were 4.35, 4.07, 3.90, 3.88, 3.40, 3.77, respectively, at the week 0, which was coincident with the actual water quality. The CPV of the stations 0 and I, the most two heavily polluted stations, continuously decreased in the process of restoration, which indicated that CPV could have commendably reflected the variables of water quality. The degressive tendency of CPV in stations II, III, V was obvious as well. The CPV of station IV was stable and its fluctuation range was ±0.15. At the end of restoration, the CPV of each station was close to that of station IV, their discrepant values were ±0.10, less than the fluctuation range of station IV.

4.6. Heterotrophy index (HI)

HI could better reflect the improvement of water quality because HI of the tested communities in each station
decreased gradually during the period of restoration (Fig. 8). Although with a little fluctuant in the process, each station’s HI was reached or close to the level of station IV at the fifth week. HI of station IV was stable and its fluctuant range was low.

4.7. Similarity coefficient and cluster analysis

Community similarity is classified as between- and within-sample similarity. The between-sample similarity coefficient should be not greater than within-sample similarity. The sample number for calculating the coefficient within sample should be more than three, if a between-sample similarity coefficient reaches or exceeds within-sample similarity

<table>
<thead>
<tr>
<th>Station</th>
<th>Week</th>
<th>Phytomastigophorans</th>
<th>Zoomastigophorans</th>
<th>Sarcodines</th>
<th>Ciliates</th>
<th>Total</th>
</tr>
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<tr>
<td>0</td>
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<td>14</td>
<td>23</td>
<td>5</td>
<td>12</td>
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<td>I</td>
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<td>22</td>
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<td>39</td>
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</tr>
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<td>3</td>
<td>5</td>
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<tr>
<td>V</td>
<td>12</td>
<td>4</td>
<td>10</td>
<td>20</td>
<td>46</td>
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<td>IV</td>
<td>15</td>
<td>11</td>
<td>4</td>
<td>12</td>
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<td>9</td>
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<tr>
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<td>1</td>
<td>12</td>
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<tr>
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<td>13</td>
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<tr>
<td>Total species</td>
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<td>25</td>
<td>18</td>
<td>57</td>
<td>133</td>
<td></td>
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</tbody>
</table>

![Fig. 3. Variables of species number for the six stations during restoration.](image-url)
coefficient, indicating that the tested samples is from the same sites (Smith et al., 1990).

In the present research, the similarity coefficients among the six samples of station IV from week 0 to week 5 were the within-sample similarity, here the average within sample similarity was 0.33. The variables of the similarity coefficients between station IV and other stations during the restoration period is shown in Fig. 9. In a whole, the similarity between tested communities and station IV gradually increased with the restoration time, till it reached or exceeded the average of within-sample similarity.

According to formula (4), similarity coefficients between any two PFU communities of the six stations were calculated at each week, based on which a cluster analysis was made (Fig. 10).
Before restoration (week 0), the most dissimilar from the main cluster was station 0, and the most similar sites were station IV and station V. station I and station II formed another small subcluster. Station III formed a cluster with stations IV and V, suggesting water quality of station III was more adjacent to slightly polluted water. With the process of restoration, stations II, I and 0 were formed in the order of smallest or smaller clusters with station IV, respectively. From week 0 to week 5, the lowest coefficient increased from 0.11 to 0.27 (Fig. 10a, f), and the highest from 0.23 to 0.34 (Fig. 10a, f). D-value decreased from 0.12 to 0.07, indicating that the tested communities became more and more similar.

4.8. Restoration velocity deduced from the community parameters variables

When clean water replaced the five tested waters with the same speed, theoretically, the most severely polluted station 0 needs the longest time to restore, in turn, the most slightly polluted station V needs the shortest time to restore. As we discuss above, CPV, HI and similarity coefficient could better reflect the improvement process of water quality. According to the changes of the three community parameters, the restoration time needed for the tested stations is analyzed as the following:

Station V. CPV was lower than that of synchronous station IV at the second week, HI was already lower than station IV at the first week. Similarity coefficient with station IV was 0.33, equal to the within-sample similarity coefficient. Thus, it is concluded that station V was restored before one week when water substitution rate was 48.93% or less.

Station III. CPV was 3.39 at the second week, very close to the 3.32 of synchronous station IV. HI (107.6) had been a little lower than that of the synchronous station IV (116.0) at the fourth week. Similarity coefficient with station IV was 0.37 at the second week, higher than within-

Fig. 10. Cluster analysis for the PFU community matrix at each week of restoration: (a) the week before restoration; (b) the first week; (c) the second week; (d) the third week; (e) the fourth week and (f) the fifth week.
sample similarity coefficient. It is concluded that station III was restored at the third week or earlier. At that time the water substitution rate was 86.68%.

Station II. CPV (3.38) was close to station IV (3.32) and HI (103.27) was just a little higher than that of station IV (100.47) at the third week. Similarity coefficient with station IV was 0.34, basically equal to the within-sample similarity. Therefore, station II had been restored at the third week at the water substitution rate of 86.68%.

Station I. CPV decreased to 3.43, close to the 3.35 of synchronous station IV at the fifth week. HI was 100.04 at the fourth week, already a little lower than that of 116.0 of station IV. Similarity coefficient with station IV was already 0.30 close to 0.33, and reached 0.39 at the fourth week. Obviously, station I could be restored at the fourth week with the water substitution rate of 93.20%.

Station 0. CPV (3.38) approached station IV (3.35) at the fifth week. HI (109.9) was a little lower than synchronous station IV (116.08) at the fourth week. Similarity coefficient with station IV was 0.33, just equal to the similarity coefficient within sample. It is concluded that station 0 was restored between the fourth week and the fifth week. HI (109.9) was a little lower than synchronous station IV at the fifth week. HI was 100.04 of station IV. Similarity coefficient with station IV was 0.33, just equal to the within-sample similarity coefficient. It is concluded that station III was restored at the third week or earlier. At that time the water substitution rate was 86.68%.

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According to the analysis between the restoration time of communities parameters with the water substitution rate, the results are shown in Table 3.

### Table 3

<table>
<thead>
<tr>
<th>Stations</th>
<th>0</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>V</th>
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<tr>
<td>Restoration time (week)</td>
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<td>4</td>
<td>3</td>
<td>&lt;3</td>
<td>&lt;1</td>
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<tr>
<td>Water substitution (%)</td>
<td>95</td>
<td>90</td>
<td>&gt;85</td>
<td>85</td>
<td>50</td>
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</table>

**5. Discussion**

In this study, we designed a particular program of using natural clean water for the reappearance of polluted water to reappear an ecological restoration succession process. Small-scale manipulative field experiments can provide a measure of the impacts that environmental variables bring to communities. The development of the methods used to measure local-scale factors for the important to restoration and management of natural water systems.

In the course of healthy community slowly restoring the damaged communities, of the determined community parameters, HI (Fig. 7) and CPV (Fig. 8) could well and truly reflected water improvement. The results of cluster analysis (Fig. 10) based on the community similarity coefficient indicated that the community structure tended to become more and more similar with the tenor of restoration. As a whole, the community structure of most heavily polluted stations 0 and II required longer time to attain a certain similarity with station IV.

Generally, tolerant species form the dominant population and develop higher individual abundance under the condition of organic pollution (Xu et al., 2005). Species number of heavily polluted stations 0 and I increased greatly at the early stage of restoration and reached the highest at the second and third week, respectively (Fig. 3). The reasons for this is, in the process of clean water replacing polluted water, clean water species and polluted water species mixed together continuously, some clean water species had a certain tolerance to pollution and the polluted water species would not disappear immediately, which made the species number to increase greatly. At the end of restoration, the species number decreased and approached the level of station IV.

Maglaef’s DI was impacted greatly by the variation of species number. Same to the species number, the DI of stations 0 and I reached the highest at the third and second week, respectively (Fig. 6). At the second and the third week, the ecological systems of the microcosms were in an unsteady status with drastic species changes and successions. Therefore, biological diversity does not have an inevitable relationship with the ecological stability (Conell, 1978; Washington, 1984), the former denotes the community structure, and the latter reflects the community function. Hutchinson (1961) proposed temporal instability as an explanation for the high species diversity of phytoplankton communities. When the ecological system went into a stable status after the restoration was ready, DI of each station inclined to consistency.

As to station IV, the percentage of Phytomastigophora fluctuated also in a larger scope, from 31.11% to 45% (Fig. 4). Natural clean water of station IV was fetched every two or three days during the experiment for nearly 40 days. During this time, the species of station IV also had a seasonal succession, because it has reported that community composition can change drastically within weeks (Kelly and Chistoserdov, 2001). This is why the average similarity coefficient of within-sample was just 0.33 but not higher.

The percentage of Phytomastigophora in the microcosms increased slowly at the early stage, but quickly at the end of restoration (Fig. 5). At the fifth week, percentage of Phytomastigophora reached the highest 37.23%. It is clear that the healthy community may have a higher proportion of autotrophic component. The variable of the ratio of phytoplankton is similar to the changing trend of water quality (Wang et al., 2004). The decrease of HI (Fig. 8) also reflected the increase of autotrophic component.

In a healthy microbial community with no pollution stress, there exists certain equilibria between autophytic organisms and heterotrophic organisms. The pollution stress makes the equilibria change, which causes the pollution tolerance species increase, consequently disturbing the inner mechanism of normal community such as food chain (Clark et al., 1979). When pollution becomes heavier, the percentage of heterotrophic organism component will increase, contrarily, autophytic organism component will increase.
References


