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Underwater light source changes nitrogen and phosphorus removal pathways by *Vallisneria spinulosa* Yan growth system



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Vallisneria spinulosa Yan (*V. spinulosa* Yan) with good ability of water purification is often used for ecological restoration of polluted water bodies. However, it is difficult to survive in turbid water bodies due to the low lighting condition. This study explored the feasibility of introducing artificial underwater light source into water bodies with high turbidity to strengthen the water restoration by *V. spinulosa* Yan. Addition of underwater light source promoted the clonal reproduction ability of *V. spinulosa* Yan, thus enhancing the removal loads of total nitrogen (TN), total phosphorus (TP), and nitrate nitrogen (NO_3^- -N) by $1.60\text{--}3.43 \times 10^{-2}$, $1.49\text{--}3.49 \times 10^{-3}$, and $0.80\text{--}2.06 \times 10^{-2} \text{ g m}^{-2} \text{ d}^{-1}$, respectively. Underwater light source significantly reduced the abundance of microbial community on *V. spinulosa* Yan leaves, as well as most nitrifying bacteria (*Nitrosomonadaceae*) and denitrifying bacteria (*Nitrospira*, *Comamonadaceae*, and *Rhodocyclaceae*) in the system. But the attachment of some *Cyanophyta* (*Chloroplast* and *Cyanobacteria*) and *Photosynthetic* bacteria (*Rhodobacter*) onto the leaves and the growth of *Methylobacteriaceae* in water and sediments were promoted. Nitrogen and phosphorus removal by the growth system of *V. spinulosa* Yan without underwater light source mainly depended on the biological processes by functional bacteria, while the absorption and co-assimilation effect of *V. spinulosa* Yan with underwater light source.

Submerged plants are the important primary producers in lake ecosystems, which play an important role in water purification, structure restoration, function transformation, and health maintenance of water ecosystems^{1–3}. Due to the influence of human activities, such as the continuous output and accumulation of a large number of nitrogen and phosphorus caused by point and area-source pollution, lakes as the receiving water bodies are facing the issue of eutrophication and even hyper eutrophication^{4–6}. The use of submerged plants to repair polluted rivers and eutrophic lakes has received widespread attention⁷. As an important part of the aquatic ecosystem, submerged plants can absorb nitrogen, phosphorus, and other nutrients in the water during their growth process, thereby reducing the pollution load of nitrogen and phosphorus in water bodies. In addition, the surface of submerged plants can attach suspended solids to reduce water turbidity⁸, inhibit the release of nutrients in sediments through their roots, and also resist the

resuspension of sediments caused by wind and wave disturbances⁹. Therefore, submerged plants play an important role in ecological restoration of water bodies.

The growth of submerged plants and the ecological restoration process are influenced by many factors. Suspended solids is one of the important factors, which contains organic and inorganic components and is also a significant indicator of water quality^{10–12}. The increase of suspended solids in water bodies will change the optical properties of water bodies, such as transparency, optical attenuation coefficient, color, and turbidity of water, as well as decrease the landscape of water bodies¹³. Suspended solids can release nitrogen and phosphorus through desorption processes, which is one of the most important factors affecting lake eutrophication¹⁴. The adsorption, flocculation, deposition, and resuspension of suspended solids can also cause local pollution of water bodies. In the water bodies with high content of suspended solids, the suspended particles reduce the underwater light intensity and light availability by the effects of refraction, reflection, and

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scattering. Thus, photosynthesis of submerged plants is inhibited¹⁵, even leading to death of plants, which directly affects its ecological restoration process.

Doyle et al. found that water turbidity could significantly affect the survival and growth of *Vallisneria americana* winterbuds and seedlings, relative growth of the plants, total number of rosettes and leaves¹⁶. Submerged plants obtain lower light energy due to water turbidity or sediment resuspension. Cao et al. observed that light stress gradually reduced the electron transfer rate of PS II, and concluded that turbid water mainly changed the growth of *Vallisneria natans* by affecting the photosynthesis¹⁷. Chen et al. studied the effects of water flow and suspended solids on the surface biofilm of two submerged plants (*Myriophyllum verticillatum* and *Vallisneria natans*)⁸. They found that the main peak particle size of suspended solids (8.71–13.18 μm) in the treatment group with submerged plants was lower than that (15.14–19.95 μm) without submerged plants. The addition of water flow and suspended solids significantly increased the biofilm thickness attached to *Myriophyllum verticillatum*, but significantly reduced the biofilm thickness of *Vallisneria natans*.

In summary, it is necessary to reduce the concentration of suspended solids in water bodies for submerged plants to get sufficient light, which is beneficial for improving the growth of submerged plants and their water restoration efficiency. At present, the common method of constructing submerged plant community is cultivating submerged plants after dredging or lowering the water level, or planting them in enclosure to reduce the influence of suspended solids¹⁸. This study attempted to meet the light conditions required for the growth of submerged plants in the water with high turbidity by directly adding underwater light source around the plants.

Vallisneria plants, as one kind of typical submerged plants, are widely distributed in surface water bodies around the world. Compared with other kinds of submerged plants, they have the lowest light compensation point and the lowest demand for light^{19,20}, and can be used as a pioneer species in the water restoration process by submerged plants. In addition, the turbidity in natural water bodies is mainly caused by suspended inorganic and organic substances. Different kinds of suspended solids under similar lighting conditions may generate different influences on submerged plants and its restoration process for water bodies, and the corresponding influencing mechanism is still unclear. Therefore, this study selected the common submerged plants (*V. spinulosa* Yan) in surface water bodies as the research object. The effects of underwater light source on the ecosystem constructed by *V. spinulosa* Yan under different water turbidity conditions were explored, focusing on the physiological and biochemical characteristics of *V. spinulosa* Yan, water purification ability and microbial community composition of the ecosystem. We assume that the supplementation of underwater light source will affect the growth of *V. spinulosa* Yan and change the microbial community structure attached to the surface of *V. spinulosa* Yan. In this study, *V. spinulosa* Yan was exposed to eutrophic water containing different kinds of suspended solids under the supplementation of underwater light source. The aims were to demonstrate whether the underwater light source can improve the water purification capacity of the ecosystem, promote the growth of *V. spinulosa* Yan, and change the composition of microbial communities in the system. This study will provide a theoretical basis for the construction or restoration of submerged plants in natural eutrophic lakes.

Results

Efficiency of water purification

It can be seen from Supplementary Fig. 1 that there was no significant difference in water temperature between different experimental groups, but there were significant differences in pH, DO, and EC ($P < 0.05$). DO value in the four experimental groups with underwater light source (4.94–5.77 mg/L) was significantly higher than that without underwater light source (2.35–3.15 mg/L) ($P < 0.05$), pH had the similar trend. pH (7.23–9.03) and DO (4.06–7.90 mg/L) of the NWL were the highest, while the EC value (133.27–255.03 $\mu\text{S}/\text{cm}$) of the NW was the highest.

The removal loads of TN in the experimental groups with underwater light source in Supplementary Fig. 2 were $1.43\text{--}3.60 \times 10^{-2} \text{ g m}^{-2} \text{ d}^{-1}$ (NWL), $4.24\text{--}8.48 \times 10^{-3} \text{ g m}^{-2} \text{ d}^{-1}$ (AWL), $3.03\text{--}9.37 \times 10^{-2} \text{ g m}^{-2} \text{ d}^{-1}$ (CKL), and $4.10\text{--}11.10 \times 10^{-2} \text{ g m}^{-2} \text{ d}^{-1}$ (IWL), which were significantly higher than that without underwater light source ($P < 0.05$). There was a significant difference in TN removal load between natural water and AW, IW, and CK, but there was no significant difference among AW, IW, and CK.

Single-factor analysis showed that the presence of underwater light source and the type of suspended solids had no significant effect on the removal load of $\text{NO}_3^- \text{--N}$ in water. However, it can be seen from Supplementary Fig. 3a, c, d that in addition to the experimental group with organic suspended solids, the addition of underwater light source could enhance the removal loads of $\text{NO}_3^- \text{--N}$ in other experimental groups. In the later stage of the experiment, the difference in the removal loads of $\text{NO}_3^- \text{--N}$ between the experimental groups with and without underwater light source gradually decreased (Supplementary Fig. 3). In addition, there was a significant positive correlation between WT and the removal loads of $\text{NO}_3^- \text{--N}$ ($P < 0.05$).

Supplementary Fig. 4 illustrated that the removal loads of $\text{NH}_4^+ \text{--N}$ in water were not significantly affected by the presence of underwater light source, but there was significant difference among the water bodies with different kinds of suspended solids (NW, AW, and IW, $P < 0.05$). The removal loads of $\text{NH}_4^+ \text{--N}$ in AWL ($3.53 \times 10^{-2} \text{ g m}^{-2} \text{ d}^{-1}$), IWL ($4.27 \times 10^{-2} \text{ g m}^{-2} \text{ d}^{-1}$) and CKL ($3.99 \times 10^{-2} \text{ g m}^{-2} \text{ d}^{-1}$) were 2.5–3 times higher than that of NWL. Different types of suspended solids had little effect on the TP removal in water, but the presence of underwater light source had a significant effect (Supplementary Fig. 5). The removal loads of TP in the experimental groups with underwater light source were significantly higher than that without underwater light source ($P < 0.05$). The average removal loads of TP by AWL, IWL, and CKL was $6.80 \pm 0.80 \times 10^{-3} \text{ g m}^{-2} \text{ d}^{-1}$, but that without underwater light source was $3.94 \pm 0.17 \times 10^{-3} \text{ g m}^{-2} \text{ d}^{-1}$. In addition, the presence of underwater light source had no significant effect on the $\text{PO}_4^{3-} \text{--P}$ removal loads of the experimental groups (Supplementary Fig. 6). The $\text{PO}_4^{3-} \text{--P}$ removal loads of CKL was the highest, with an average of $6.57 \times 10^{-3} \text{ g m}^{-2} \text{ d}^{-1}$.

Physiological and biochemical characteristics of *V. spinulosa* Yan

Chla is the main photosynthetic pigment for energy conversion in plants. Depending on the different protein environment, chl a can both harvest light energy and act as a redox participant in the main charge separation of PSII and PSI^{21,22}. Chlb is the main component of the light-harvesting protein complex, and its main function is to absorb and transfer solar energy^{23,24}. The chlorophyll content of the experimental groups with underwater light source was significantly lower than that without underwater light source ($P < 0.01$), while there was no significant difference in chlorophyll content between the leaves of *V. spinulosa* Yan with different types of suspended solids. The average chlorophyll content of the experimental groups was NW (1.12 mg/g) > IW (1.09 mg/g) > AW (1.08 mg/g) > CK (1.08 mg/g) > NWL (1.03 mg/g) > CKL (0.97 mg/g) > AWL (0.91 mg/g) > IWL (0.88 mg/g).

Carotenoid is a non-enzymatic antioxidant. It can absorb different light and play the role of auxiliary pigment under light restriction, and also protect the formation of chl a and chl b²⁵. The carotenoid content of the experimental groups with underwater light source was significantly higher than that without underwater light source (Fig. 1b), while different types of suspended solids had no significant effect on it. The average carotenoid content of each experimental group was NWL (0.154 mg/g) > CKL (0.152 mg/g) > AWL (0.143 mg/g) > IWL (0.138 mg/g) > NW (0.126 mg/g) > CK (0.125 mg/g) > AW (0.124 mg/g) > IW (0.105 mg/g). Among them, the carotenoid content in the leaves of *V. spinulosa* Yan was the lowest in the experimental group containing inorganic suspended solids.

SOD can scavenge superoxide anion radicals and plays an important role in the balance of oxidation and antioxidation in plants^{26,27}. In Fig. 2a, there was no significant difference in SOD enzyme activity between the

Fig. 1 | Physiological characteristics of *V. spinulosa* Yan leaves. a Chlorophyll a + b content; **b** carotenoid content. ChlU+b represents chlorophyll a + b content. NW, AW, IW, and CK represent the natural lake water group, simulated lake water + *Microcystis aeruginosa* group, simulated lake water + kaolin group, and simulated lake water group, respectively. NWL, AWL, IWL, and CKL are the corresponding groups with underwater light source.

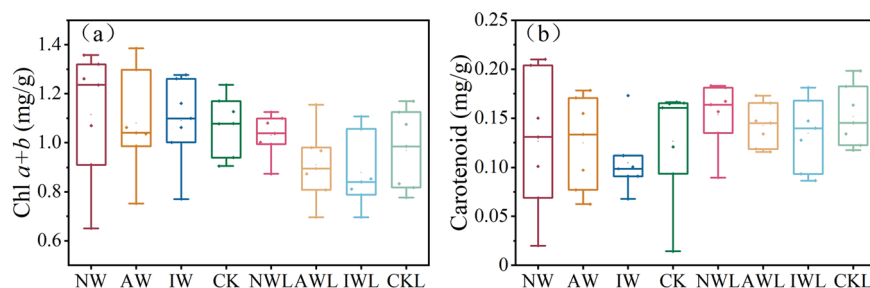
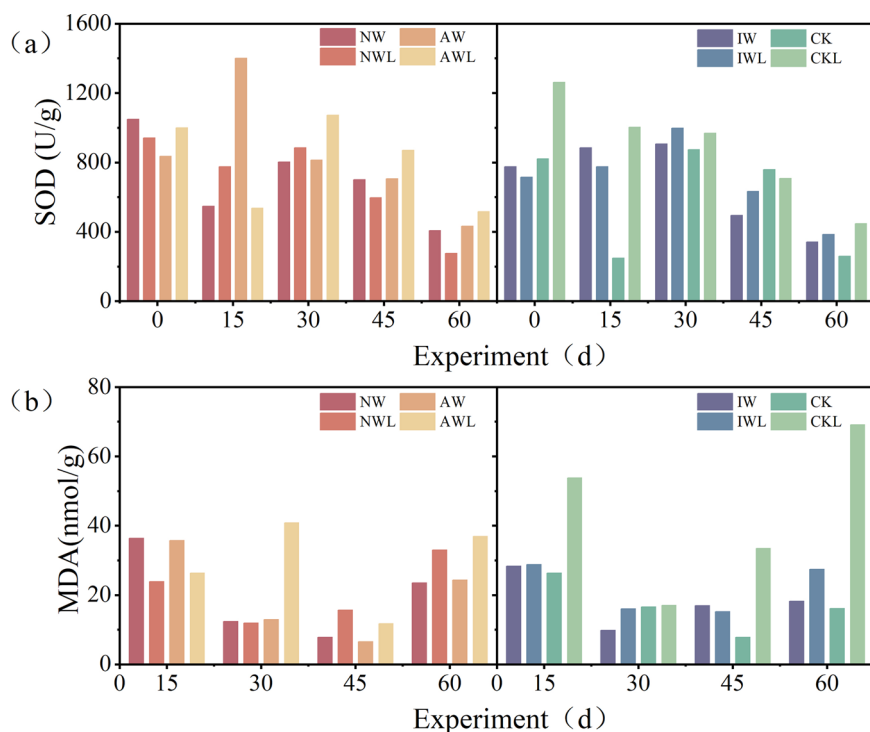


Fig. 2 | Biochemical characteristics of *V. spinulosa* Yan leaves. a SOD enzyme activity **b** MDA enzyme content. SOD and MDA represent activity of superoxide dismutase and content of malondialdehyde, respectively. NW, AW, IW, and CK represent the natural lake water group, simulated lake water + *Microcystis aeruginosa* group, simulated lake water + kaolin group, and simulated lake water group, respectively. NWL, AWL, IWL, and CKL are the corresponding groups with underwater light source.



leaves of *V. spinulosa* Yan in different experimental groups. The SOD enzyme activity in the most experimental groups was slightly increased and then decreased, and presented as a decrease trend from 30 d to 60 d. MDA is one of the products of membrane lipid peroxidation. It acts as an indicator of lipid peroxidation, indicating the degree of membrane lipid peroxidation and the response of plants to stress conditions^{28,29}. From Fig. 2b, we can see that the content of MDA in leaves of *V. spinulosa* Yan in each group decreased first and then increased. In the later stage of the experiment, the leaves of *V. spinulosa* Yan began to senescence, resulting in an increase in MDA content. Compared with the experimental group without underwater light source, the changing trend of MDA content in the experimental group with underwater light source was more obvious. The changing trend of AWL and CKL was the most obvious, and the MDA content of CKL increased rapidly as the experiment progressed.

Microbial community composition

Ace and Chao are indicators for evaluating the richness of microbial communities, and the larger Chao and Ace values indicate the more total number of microbial species^{30,31}. Compared with other regions, Ace and Chao in water of the device had the lowest values, and the leaf area had the second lowest value (Fig. 3a, b). The microbial abundance of the leaf in the experimental groups with underwater light source was lower than that without underwater light source (Fig. 3). The microbial abundance on leaf surface of the CKL group was almost the same as that in the water. The

abundance of microbial communities in the sediments and the root surface of *V. spinulosa* Yan was the highest, while it within the roots of *V. spinulosa* Yan was lower.

Shannon index can evaluate the diversity of microorganisms in samples, and both Shannon and the Simpson index are often used to reflect alpha diversity^{32,33}. The larger Shannon value and the smaller Simpson value indicate the higher community diversity. Figure 3c, d showed lower microbial diversity in water. Compared with the water area, the leaf surface of *V. spinulosa* Yan had the higher microbial diversity. The Ace, Chao, Shannon, and Simpson indexes in the leaf area indicate that the biofilm is attached to the leaf surface of *V. spinulosa* Yan, forming a suitable habitat for bacterial growth. In addition, the microorganisms on the root surface of *V. spinulosa* Yan and the sediment surface had the highest microbial diversity.

The bacteria of all samples were classified and screened at the phylum level, and the OTUs with the relative abundance of less than 1% were assigned to others. The relative abundance map of free-living bacteria at the phylum level was shown in Fig. 4. A total of 14 dominant bacterial phyla were obtained in the five regions, including *Proteobacteria*, *Actinobacteriota*, *Cyanobacteria*, *Acidobacteriota*, *Bacteroidota*, *Chloroflexi*, *Myxococcota*, *Firmicutes*, *Patescibacteria*, *Gemmatimonadota*, *Desulfobacterota*, *Nitrospirota*, *Verrucomicrobiota*, and *Methylomirabilota*.

NMDS analysis of bacteria at the OTU level showed that there were significant differences in bacterial communities between different regions

Fig. 3 | Differences of microbial alpha diversity indexes at different locations. a Chao, **(b)** Ace, **(c)** Shannon, **(d)** Simpson.

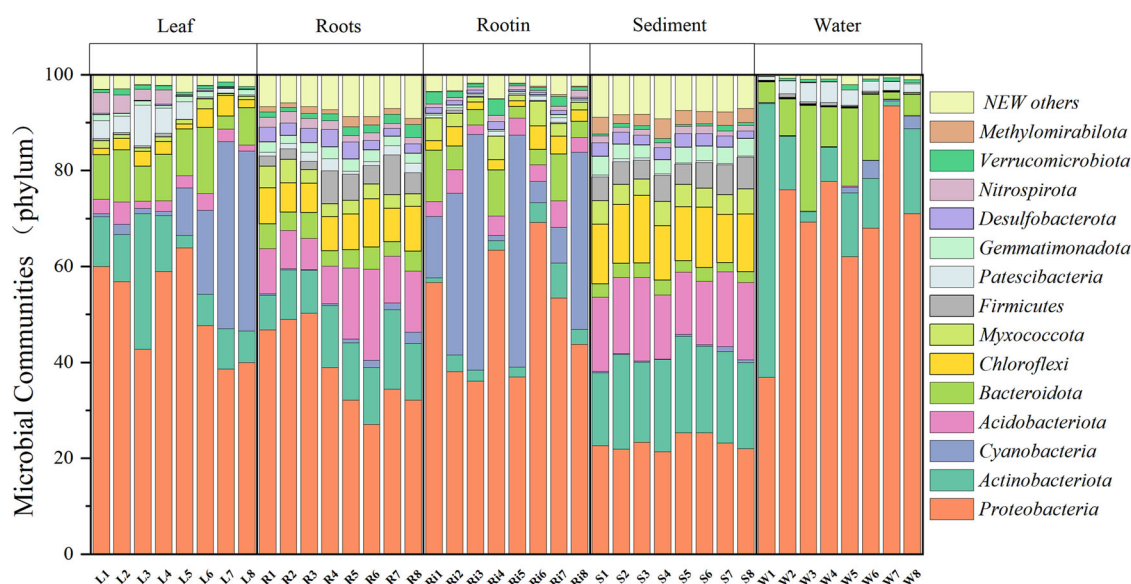
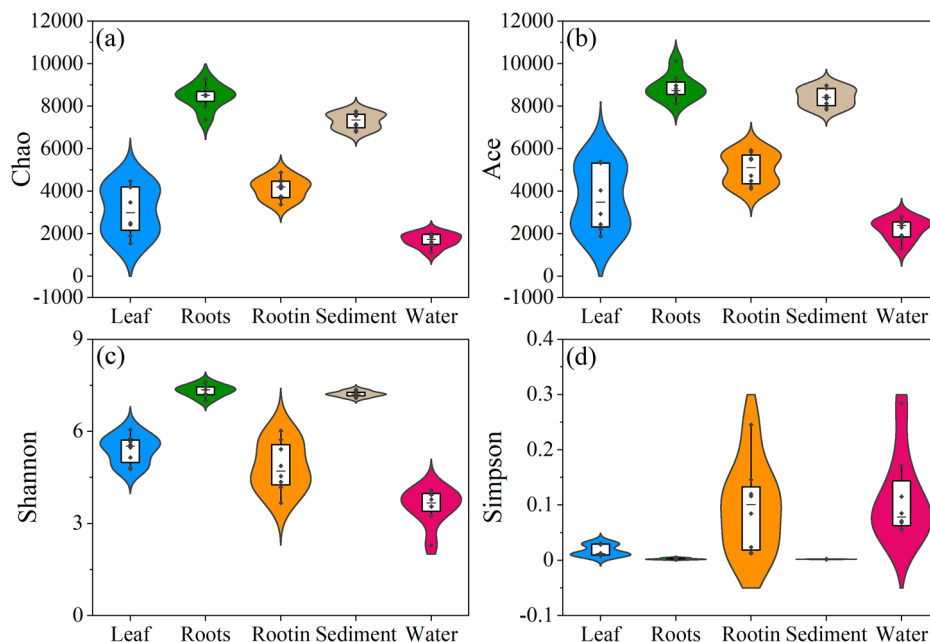


Fig. 4 | The percentage of bacterial flora in different positions of each experimental group at the level of phylum. The letters L, R, Ri, S, and W represent the microorganisms taken from the leaves of *V.spinulosa* Yan, the area around the root

system, the internal area of the root, the sediment area and the water area respectively. The numbers represent different experimental groups, 1: NW 2: AW 3: IW 4: CK 5: NWL 6: AWL 7: IWL 8: CKL.

(Fig. 5a). The Stress of NMDS analysis results is 0.1 (<0.2), indicating that the analysis results are reliable. There was almost no difference in the distribution of microorganisms in the sediments of different experimental groups, while the microorganisms inside and around the roots of *V.spinulosa* Yan were similar. In addition, the relative distance of different points in the leave surface area of *V.spinulosa* Yan is far. Therefore, the microbial community on the surface of *V.spinulosa* Yan leaves needs further analysis. Figure 5b shows that the Stress of NMDS analysis results is 0.004 (<0.05), indicating the highly representative results. The microbial community on the leaf surface of *V.spinulosa* Yan in eight different experimental groups was significantly different. Underwater light source had a great impact on the microbial community of the leave surface of *V.spinulosa* Yan in simulated water bodies.

Discussion

Addition of underwater light source promoted the photosynthesis of *V.spinulosa* Yan, which can convert more CO_2 to O_2 , resulting in the increase of pH in the water (Supplementary Fig. 1a). Pearson correlation analysis shows that there is a significant negative correlation between water temperature (WT) and dissolved oxygen (DO) ($P < 0.01$). When the ambient temperature is low, the DO content in the water increases, which may be due to a decrease in the activity of aerobic microorganisms in the water and a decrease in DO consumption in the water. Kim et al. observed that nitrification was slowly processed at low temperatures in winter (the reaction rate was about half of that in summer), and nitrification activity could be controlled by adjusting DO to obtain better water quality in effluent³⁴.

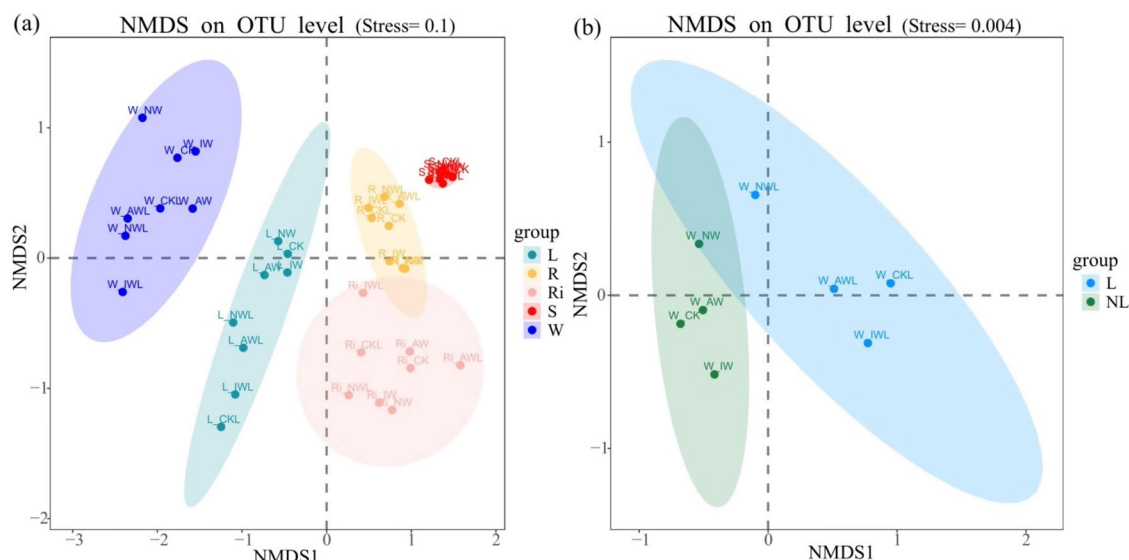
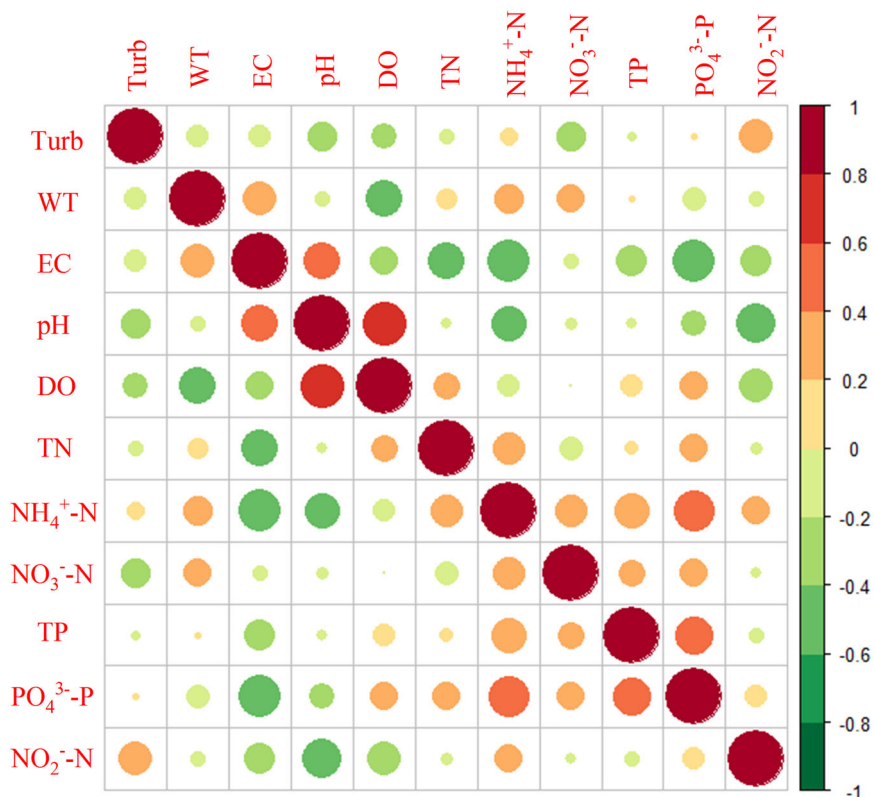


Fig. 5 | NMDS analysis of bacteria in leaf area. a Different positions; **b** different light conditions (L is underwater light source, NL is no underwater light source). Stress: Test the results of NMDS analysis. It is generally believed that when stress

<0.2, it can be expressed by the two-dimensional point diagram of NMDS, and the diagram has certain explanatory significance; When stress <0.1, it can be considered as a good sort; when stress <0.05, it has a good representativeness.

Fig. 6 | Correlation heat map between environmental factors. Turb, WT, EC, DO, TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, TP, $\text{PO}_4^{3-}\text{-P}$, and $\text{NO}_2^-\text{-N}$ represent turbidity, water temperature, conductivity, dissolved oxygen, total nitrogen, ammonia nitrogen, nitrate nitrogen, total phosphorus, phosphate, and nitrite nitrogen, respectively.



Further analysis shows that there is a significant positive correlation between WT and $\text{NO}_3^-\text{-N}$ removal load ($P < 0.05$). When WT decreased, the $\text{NO}_3^-\text{-N}$ removal loads of the experimental group decreased significantly, especially in the experimental groups with underwater light source. In addition, when WT decreases, the difference between the $\text{NO}_3^-\text{-N}$ removal loads of the experimental groups with the underwater light source and without the underwater light source becomes smaller (Supplementary Fig. 3).

It is worth noting that there is a significant positive correlation between $\text{NH}_4^+\text{-N}$ removal loads and WT, TN removal loads ($P < 0.05$) (Fig. 6).

When WT decreased, the $\text{NH}_4^+\text{-N}$ removal loads of the experimental groups decreased significantly. The activity of aerobic microorganisms may be reduced with the decrease of WT, as well as the activity of aerobic nitrifying bacteria, which may be a major reason for the reduction of $\text{NH}_4^+\text{-N}$ removal loads in water. There is a significant positive correlation between the removal loads of TN and the removal load of $\text{NH}_4^+\text{-N}$, but there is no significant difference in the removal loads of $\text{NH}_4^+\text{-N}$ between each experimental group, and the removal efficiencies achieved approximately 100%. Therefore, the difference in TN removal is mainly due to the removal of $\text{NO}_3^-\text{-N}$ rather than the removal of $\text{NH}_4^+\text{-N}$.

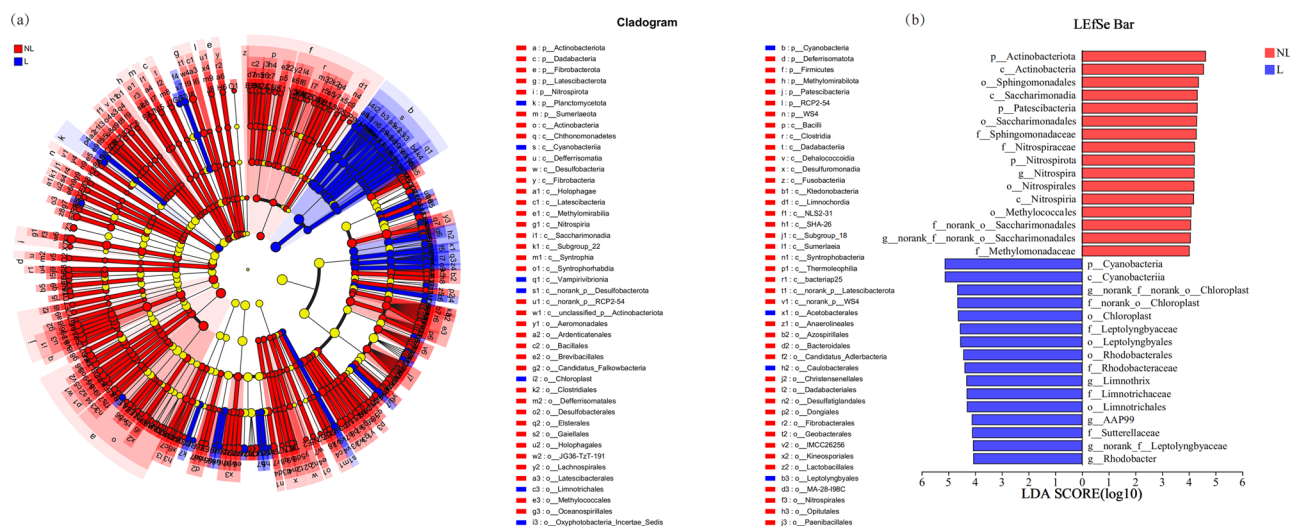


Fig. 7 | Microbial analysis on the leaf surface of *V.spinulosa* Yan under different conditions. a Branching diagram, LEfSe multi-level species analysis of microbial abundance on the leaf surface of *V.spinulosa* Yan under different light conditions; **b** Highly differential taxonomy and bacteria with linear discriminant analysis (LDA) values greater than 4. NL and L represent the groups without and with underwater light source.

The results of chlorophyll content reveal that *V.spinulosa* Yan can adapt to the water environment with low light intensity by enhancing its own chlorophyll content and strengthening its own photosynthetic organs. While in the water environment with high light intensity after adding underwater light source, *V.spinulosa* Yan will choose priority cloning and reproduction to increase its biomass. Previous studies demonstrated that higher content of carotenoids in plants was obtained when they were exposed to higher light intensity³⁵. This is consistent with the results of this study (Fig. 1b), revealing that the content of carotenoids in the experimental groups with underwater light source is higher than that without underwater light source. In addition, the content of carotenoids in the leaves of *V.spinulosa* Yan was the lowest in the water containing inorganic suspended solids. It may be due to that inorganic suspended solids have stronger scattering of light and are more easily adsorbed on the leaves of *V.spinulosa* Yan, leading to the decrease of light intensity and the inhibition on the photosynthesis of *V.spinulosa* Yan.

The SOD values in the leaves of most experimental groups were slightly increased and then decreased. The enzyme activities of *V.spinulosa* Yan in almost all the experimental groups exhibited a downward trend during 30–60 d. It indicates that *V.spinulosa* Yan is subjected to a certain degree of stress during this period, which exceeds its tolerance range and destroys its protective system, resulting in a high accumulation of reactive oxygen species and peroxidation of membrane lipid in the plants, and an increase in the MDA content (Fig. 2b). More obvious changing trend was obtained in AWL and CKL. The MDA content of CKL increased rapidly with the time, indicating that the addition of underwater light source accelerates the stress of *V.spinulosa* Yan. The reason may be that the algae adsorbed on the leaf surface of *V.spinulosa* Yan compete with *V.spinulosa* Yan for a favorable growth environment, which is not conducive to *V.spinulosa* Yan. This agrees with Song et al.'s conclusion³⁶.

The microorganisms on the leaf surface of *V.spinulosa* Yan were analyzed by LEfSe, aiming to find out the microorganisms with significant differences between the experimental groups with and without underwater light source, and the microorganisms with >4 of LDA values were listed (Fig. 7). The branch bacteria with a higher abundance on the leaf surface of *V.spinulosa* Yan without underwater light source included *c_Actinobacteria*, *f_Saccharimonadales*, *c_Sphingomonadales*, *g_Nitrospira*, *f_Methylomonadaceae*. Interestingly, both anoxic (*f_Methylomonadaceae*) and aerobic (*g_Nitrospira*) microorganisms existed on the leaf surface of *V.spinulosa* Yan under the insufficient light conditions. It indicates that biofilm with a perfect structure has been formed on the leaf surface, and the biofilm has the

dominant bacteria related to water purification. *f_Methylomonadaceae* belongs to *o_Methylococcales*, which is a methane-oxidizing bacteria that can remove greenhouse gas (CH₄). *g_Nitrospira* plays an important role in biological nitrification process, which can completely convert NH₄⁺-N into NO₃⁻-N. In addition, there were also some genus of *c_Sphingomonadales* with significant special abundance, which have the potential to improve plant growth by producing hormones for plant growth under stress conditions such as drought, salinity, and heavy metals in agricultural soils³⁷.

The branching bacteria with higher abundance on the leaf surface of *V.spinulosa* Yan with underwater light source included *p_Cyanobacteria*, *o_Chloroplast*, *f_Leptolyngbyaceae*, *g_Limnithrix*, *g_Rhodobacter*, *f_Sutterellaceae*, and *g_AAP99*. The results showed that the addition of underwater light source promoted the attachment and growth of *p_Cyanobacteria* on the leaf surface of *V.spinulosa* Yan, and there is also a close relationship between *f_Sutterellaceae* and bloom of *Microcystis aeruginosa*. Although the attachment of algae may have a negative impact on the growth of *V.spinulosa* Yan, previous studies also found that bacteria such as *f_Leptolyngbya* and *g_Limnithrix* had a high removal rate for organic matter, N, and P. Especially in treating the wastewater from distilleries, they could achieve a high removal rate of dissolved chemical oxygen demand (up to 97.4%). Interestingly, underwater light source promoted the growth of *g_Rhodobacter* on the leaf surface, which belongs to nitrogen-fixing red bacteria (Photosynthetic bacteria). Photosynthetic bacteria can use organic acids with low molecular weight (e.g., butyric acid and acetic acid) and carbohydrates (e.g., glucose and other easily hydrolyzed organic matters) to produce biohydrogen through photofermentation.

It was found that the abundance of *f_Methyloligellaceae* in the sediment increased significantly in the system with underwater light source (Fig. 8). *f_Methyloligellaceae* is one kind of methanotrophs, which can use monocarbon organic matter as source of carbon and energy. It indicates that the system with underwater light source may have stronger degradation ability of monocarbon organic matter such as methane. We also found that the abundance of *f_Methylphilaceae* increased significantly in the water with underwater light source (Fig. 9a). *f_Methylphilaceae* belongs to *c_Betaproteobacteria*, they can metabolize methylated compounds, especially methanol.

More ammonia-oxidizing bacteria (AOB) were observed on the leaf surface of *V.spinulosa* Yan without underwater light source (Fig. 10a). For *g_Nitrosomonas*, its abundance achieved 0.23–0.52% in the simulated lake

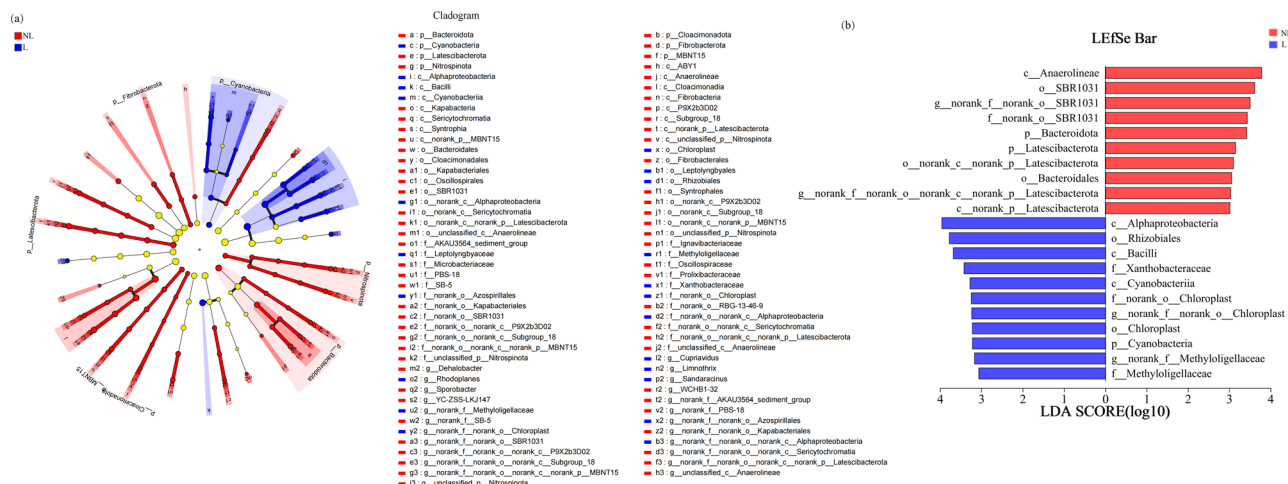


Fig. 8 | Microbial analysis in the sediment of the systems under different conditions. a Branching diagram, LEfSe multi-level species analysis of microbial abundance on sediments under different light conditions; **b** Highly differential taxonomy and bacteria with linear discriminant analysis (LDA) values greater than 3. NL and L represent the groups without and with underwater light source.

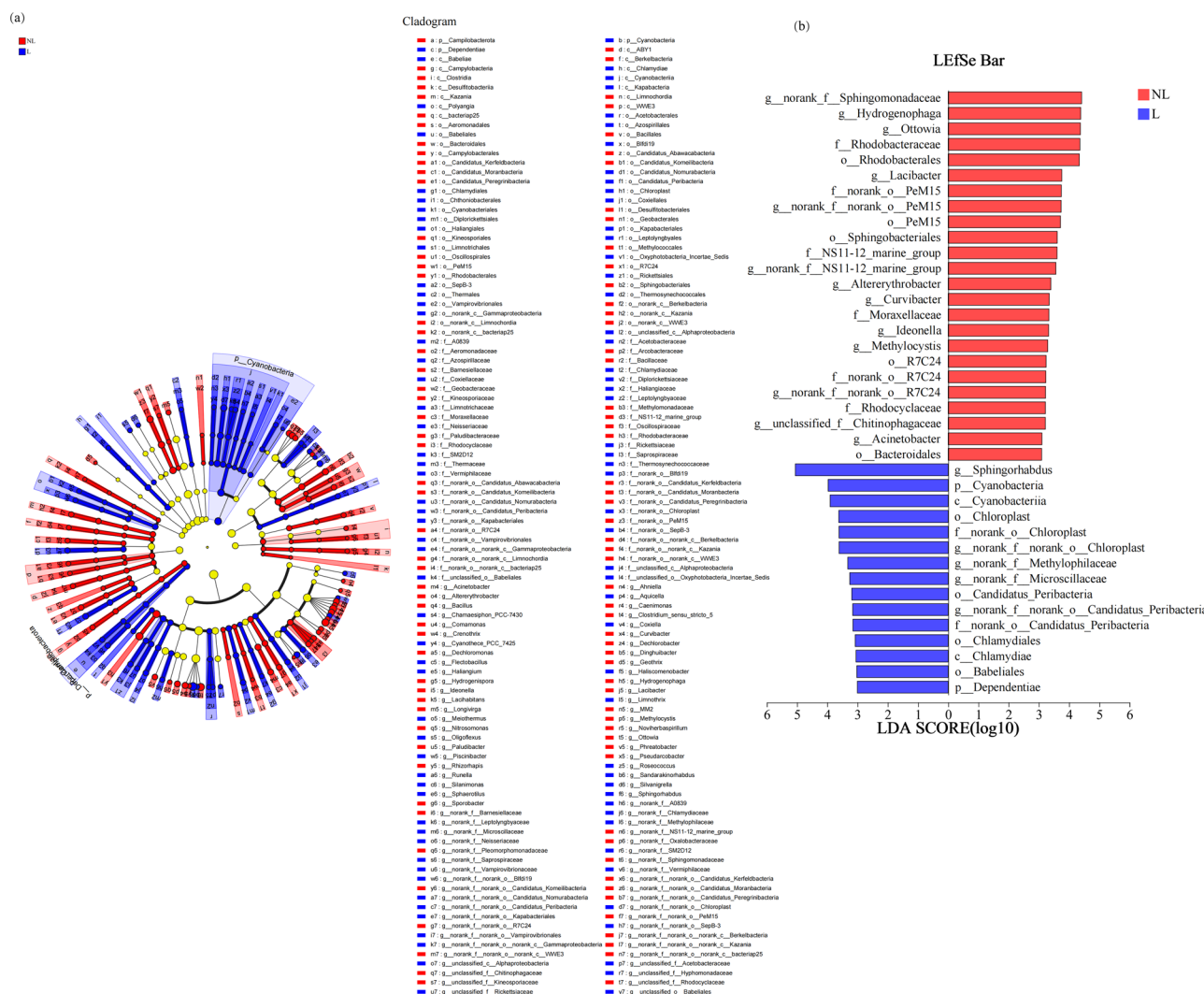


Fig. 9 | Microbial analysis in the water of the systems under different conditions. a Branching diagram, LEfSe multi-level species analysis of microbial abundance in water under different light conditions; **b** Highly differential taxonomy and bacteria with linear discriminant analysis (LDA) values greater than 3. NL and L represent the groups without and with underwater light source.

Fig. 10 | Relative abundance of nitrogen removal bacteria in different locations. a AOB; b NOB; c DB. AOB, NOB, and DB represent ammonia-oxidizing bacteria, nitrite-oxidizing bacteria, and denitrifying bacteria. NW, AW, IW, and CK represent the natural lake water group, simulated lake water + *Microcystis aeruginosa* group, simulated lake water + kaolin group, and simulated lake water group, respectively. NWL, AWL, IWL, and CKL are the corresponding groups with underwater light source.

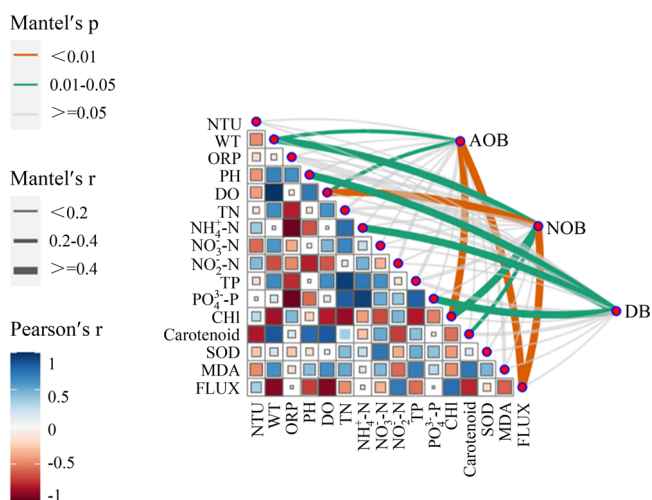
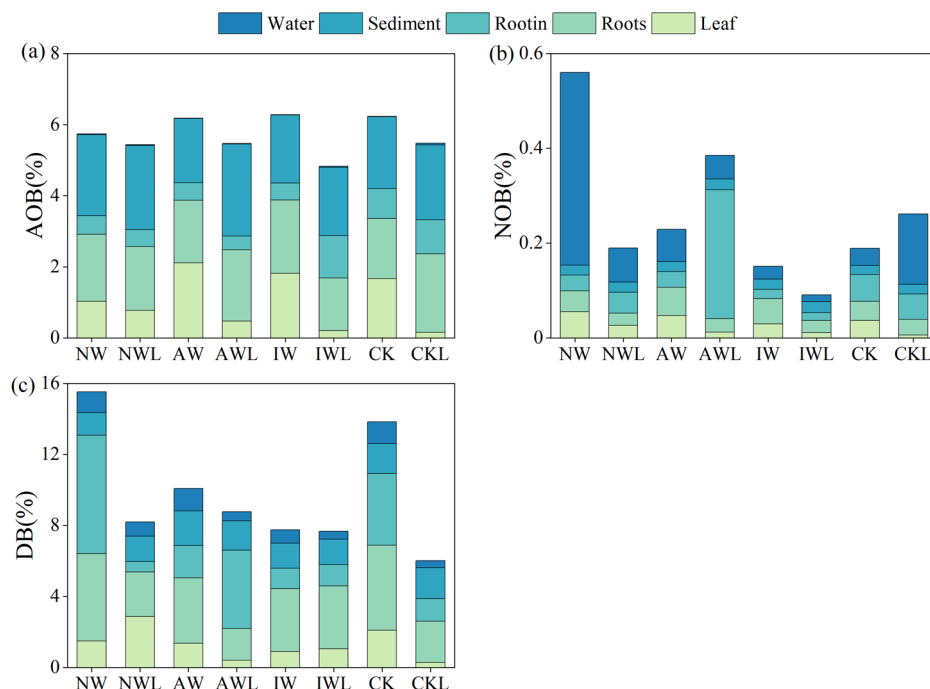


Fig. 11 | Heatmap of correlation between environmental variables and abundance of denitrification species/functional bacteria. AOB, NOB, and DB represent ammonia-oxidizing bacteria, nitrite-oxidizing bacteria, and denitrifying bacteria. NTU, WT, ORP, DO, TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, TP, $\text{PO}_4^{3-}\text{-P}$, $\text{NO}_2^-\text{-N}$, CHI, SOD, and MDA represent turbidity, water temperature, redox potential, dissolved oxygen, total nitrogen, ammonia nitrogen, nitrate nitrogen, total phosphorus, phosphate, nitrite nitrogen, chlorophyll, activity of superoxide dismutase, and content of malondialdehyde respectively.

water and 0.05% in the natural lake water without underwater light source, while its abundance was less than 0.1% on the leaf surface of *V. spinulosa* Yan with underwater light source. In the simulated lake water with low content of suspended solids, the abundance of *g_Nitrosomonas* reached 0.42% (without underwater light source) and 0.29% (with underwater light source) in the root interior of *V. spinulosa* Yan. The abundance of *f_Nitrosomonadaceae* in the water without underwater light source was 2.1–1.02%, achieving the highest abundance in AW and the lowest abundance in NW. Its abundance in the water with underwater light source reached 0.15–0.7%, with the highest abundance in NWL and the lowest abundance in CKL. In

the sediment, the abundance of *f_Nitrosomonadaceae* in each group was AWL (2.59%) > NWL (2.37%) > CKL (2.10%) > IWL (1.91%), NW (2.28%) > CK (2.02%) > IW (1.91%) > AW (1.81%). Therefore, the addition of underwater light source promoted its abundance in the sediment. In addition, the abundance of nitrite-oxidizing bacteria (NOB) on the leaf surface of *V. spinulosa* Yan without underwater light source was higher than that with underwater light source (Fig. 10b). The abundance of NOB such as *g_Nitrospira* on the leaf surface of each group was NW (4.22%) > AW (3.80%) > IW (2.30%) > CK (2.90%) > AWL (0.33%) > NWL group (0.18%) > CKL (0.10%) > IWL (0.09%).

The abundance of denitrifying bacteria (DB) in the sediment and the root surface of *V. spinulosa* Yan exhibits a significant correlation with the presence or absence of underwater light source (Fig. 10c). The main DB in the system included *g_Bacillus*, *g_Paracoccus*, *g_Methylobacterium*, *f_Alcaligenaceae*, *g_Pseudomonas*, the dominant DB with acetate as carbon source (*f_Comamonadaceae*, *f_Rhodocyclaceae*), and with methanol as carbon source (*g_Methylophilus*, *g_Methylobacillus*, *g_Hyphomicrobium*). We found that the abundance of *f_Comamonadaceae* was significantly reduced on the leaf surface of *V. spinulosa* Yan with underwater light source. It may be due to the increase of DO around *V. spinulosa* Yan by supplying sufficient light. Interestingly, except for the root interior of *V. spinulosa* Yan, the abundance of *f_Rhodocyclaceae* in other four regions is significantly correlated with the presence or absence of underwater light source. The addition of underwater light source decreased the abundance of *f_Rhodocyclaceae* in the leaves and root surface of *V. spinulosa* Yan, sediment surface, and water. The abundance of *g_Paracoccus* in the water (1.42%) was higher than that in root surface (0.08%) and sediment (0.02%) with underwater light source, and the highest content (2.24%) was observed on the leaf surface of *V. spinulosa* Yan in NWL. But it had the highest abundance (2.38%) on the leaf surface of *V. spinulosa* Yan without underwater light source. However, the addition of underwater light source promoted the growth of *g_Bacillus* in the sediment and on the leaf surface of *V. spinulosa* Yan.

The above analysis showed that the experimental groups without underwater light source had a higher abundance of AOB, especially in the leaf surface of *V. spinulosa* Yan (Fig. 10a). The abundance of NOB in the leaf surface, root interior and surface, and water (except for the water in CK) without underwater light source was higher than that with underwater light source (Fig. 10b). In addition, the abundance of NOB and AOB is

Fig. 12 | Relative abundance of phosphorus removal bacteria in different locations. **a** PAOs; **b** GAOs. PAOs and PAOs are phosphorus-accumulating organisms and glycogen-accumulating organisms. NW, AW, IW, and CK represent the natural lake water group, simulated lake water + *Microcystis aeruginosa* group, simulated lake water + kaolin group, and simulated lake water group, respectively. NWL, AWL, IWL, and CKL are the corresponding groups with underwater light source.

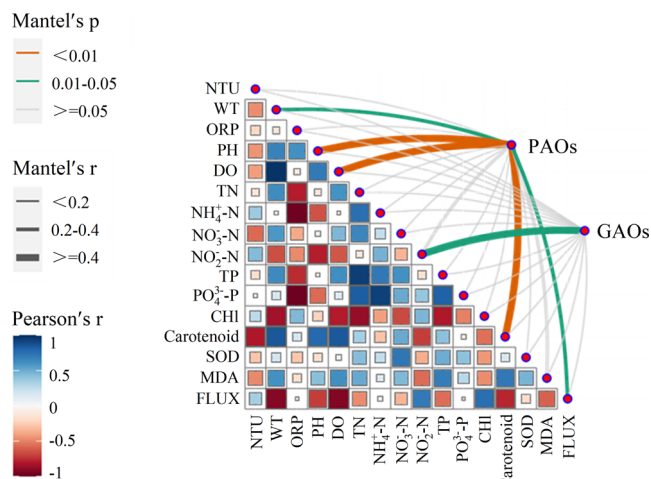
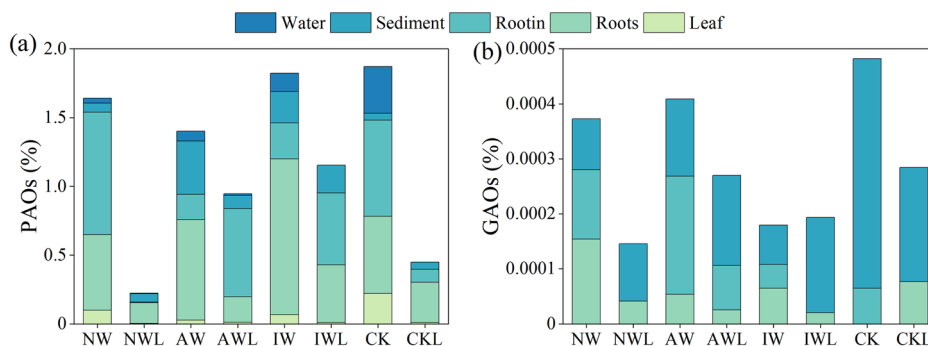


Fig. 13 | Heatmap of the correlation between environmental variables and the abundance of P removal species/functional bacteria. PAOs and PAOs are phosphorus-accumulating organisms and glycogen-accumulating organisms. NTU, WT, ORP, DO, TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, TP, $\text{PO}_4^{3-}\text{-P}$, $\text{NO}_2^-\text{-N}$, CHI, SOD, and MDA represent turbidity, water temperature, redox potential, dissolved oxygen, total nitrogen, ammonia nitrogen, nitrate nitrogen, total phosphorus, phosphate, nitrite nitrogen, chlorophyll, activity of superoxide dismutase, and content of malondialdehyde respectively.

significantly negatively correlated with the content of $\text{NO}_2^-\text{-N}$ and $\text{NH}_4^+\text{-N}$ in the water (Fig. 11). But there is no significant difference in the removal loads of $\text{NH}_4^+\text{-N}$ in the water between with and without underwater light source (Supplementary Figs. 3 and 4), indicating that the removal of $\text{NH}_4^+\text{-N}$ in the groups without underwater light source is closely related to nitrifying bacteria, while the removal of $\text{NH}_4^+\text{-N}$ in the groups with underwater light source may not be mainly dependent on nitrifying bacteria. Similar phenomena were found between DB and $\text{NO}_3^-\text{-N}$ (Figs. 10c, 11), indicating that the removal of $\text{NO}_3^-\text{-N}$ in the groups without underwater light source is closely related to DB, while the removal of $\text{NO}_3^-\text{-N}$ in the groups with underwater light source may not be mainly dependent on DB. Previous studies pointed out that there is a relationship between microalgae and nitrification-related bacteria that promotes the removal of N and P in water or competes with each other. Under insufficient $\text{NH}_4^+\text{-N}$, there is a competitive relationship between microalgae and nitrification-related bacteria³⁴, and sufficient light source promotes the growth of microalgae and inhibit the growth of nitrification-related bacteria³⁸. Figure 7 showed that the addition of underwater light source promoted the attachment and growth of algae bacteria on the leave surface of *V.spinulosa* Yan. Therefore, in the groups with underwater light source, the removal of N may mainly depend on the assimilation of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ by the growth of algae and *V.spinulosa* Yan, while in the groups without underwater light source, the

removal of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ mainly depends on the nitrifying bacteria (AOB and NOB) and DB.

The removal of phosphorus by microorganisms mainly depends on phosphorus-accumulating organisms (PAOs) with phosphorus-accumulating ability and glycogen-accumulating organisms (GAOs) without phosphorus-accumulating ability. The main PAOs in the groups were *g_Accumulibacter* and *g_Dechloromonas* and the main GAOs were *g_Defluviicoccus* and *g_Candidatus_Competibacter*. The abundance of PAOs on the root surface of *V.spinulosa* Yan was IW (1.13%) > AW (0.73%) > CK (0.56%) > NW (0.55%) > IWL (0.42%) > CKL (0.30%) > AWL (0.18%) > NWL (0.15%). The abundance of PAOs in the sediment was AW (0.39%) > IW (0.23%) > NW (0.07%) > CK (0.05%), IWL (0.20%) > AWL (0.10%) > NWL (0.06%) > CKL (0.05%) (Fig. 12). The addition of underwater light source reduced the abundance of PAOs in each group, especially on the root surface. Figure 13 shows that there is a significant positive association between DO and PAOs. At the same time, in the experimental groups without underwater light source, the TP removal rate and DO increased gradually in the later stage of the experiment (Supplementary Figs. 1 and 5). It may be due to that the increase of the abundance of PAOs in the system promotes the absorption of P under aerobic conditions. The TP removal is not related to PAOs and GAOs with underwater light source ($P > 0.05$), but the TP removal load was significantly higher than that without underwater light source (Supplementary Fig. 5). Therefore, the experimental groups with underwater light source mainly relies on the growth of microalgae and *V.spinulosa* Yan in the system. Compared with the other experimental groups, the group with inorganic suspended solids had a higher abundance of PAOs in each region. It may be that inorganic suspended solids can act as good carrier for attachment and growth of PAOs, and will not compete with PAOs for growth environment and nutrients, thereby promoting the growth of PAOs. The addition of underwater light source in the water with inorganic suspended solids can promote the growth of *V.spinulosa* Yan, and can also maintain a high abundance of PAOs in the system.

In real turbid water bodies, growth of *V.spinulosa* Yan is usually inhibited due to the low lighting intensity, especially in deep water areas. As a result, its ecological restoration effect for water bodies is significantly reduced. This study suggests addition of underwater light source around *V.spinulosa* Yan during its growth stage, aiming to improve its growth. After about two months, *V.spinulosa* Yan grows to a certain height when it can receive natural light, the underwater light source can be removed. During the two-month stage, the cost for adding underwater light source mainly comes from purchasing light source and electricity consumption. Based on this study, the former is about USD 15.76/(m² water area), and the latter is approximately USD 2.24/(m² water area). It is worth noting that the underwater light source can be removed from water bodies and reused in other ecological restoration projects after *V.spinulosa* Yan can receive natural light. Moreover, solar energy is suggested to electricity supply for the underwater light source. Therefore, the cost can be significantly decreased in real projects. In addition, installation and removal of the light source from water bodies can be achieved by using some ropes, suggesting convenient

Table 1 | Experimental setting for different groups

Group number	Settings	Type of suspended solids
NW	Natural lake water	Mixed suspended solids
NWL	Natural lake water + underwater light source	Mixed suspended solids
AW	Simulated lake water + <i>Microcystis aeruginosa</i>	Organic suspended solids
AWL	Simulated lake water + <i>Microcystis aeruginosa</i> + underwater light source	Organic suspended solids
IW	Simulated lake water + kaolin	Inorganic suspended solids
IWL	Simulated lake water + kaolin + underwater light source	Inorganic suspended solids
CK	Simulated lake water	No suspended solids
CKL	Simulated lake water + underwater light source	No suspended solids

operation. In conclusion, field scale application of adding underwater light source to improve the growth of *V. spinulosa* Yan and its ecological restoration effect is feasible.

Methods

Experimental materials

V. spinulosa Yan used in this study was taken from a natural lake. The growth substrate was the planting soil in an ecological botanical garden, the gravels are removed by sieves and mixed well for later use. The water used in the experiment contained both natural and simulated lake water. The water quality was TN 2.0 mg/L, $\text{NH}_4^+\text{-N}$ 1.0 mg/L, $\text{NO}_3^-\text{-N}$ 1.0 mg/L, TP 0.2 mg/L. The underwater light source was a 7 W LED lamp. NH_4Cl , KNO_3 , K_2HPO_4 , and kaolin used to simulate lake water with different kinds of suspended solids were purchased from the National Pharmaceutical Group Chemical Reagents Co., Ltd. (China), and their purity was analytical purity.

Experimental design

The experiment was carried out in an ecological botanical garden of Wenzhou University (Zhejiang Province, China). The growth system of *V. spinulosa* Yan was established in a device made of polypropylene (length $\times$ width $\times$ height = 70 cm $\times$ 75 cm $\times$ 80 cm), which was divided into three independent units (length $\times$ width $\times$ height = 70 cm $\times$ 25 cm $\times$ 80 cm) set as parallel groups. In each unit, 18 plants of *V. spinulosa* Yan were planted in the soil with the layer height of 10 cm. After the cultivation and stable growth of *V. spinulosa* Yan with 15 d, the experiment was started and lasted for 60 d. Hydraulic retention time (HRT) and water depth was set as 4 d and 40 cm. Because the photosynthetically active radiation received by *V. spinulosa* Yan in the bottom of the device was close to 0, the artificial light source was added and fixed on the inner wall of the device and located at 10 cm above the soil layer. The light supplement time was 6: 00 am–6: 00 pm every day. Eight experimental groups were set to stimulate different growing conditions for *V. spinulosa* Yan, the specific settings were shown in Table 1. *Microcystis aeruginosa* and kaolin were used to simulate organic and inorganic suspended solids according to previous studies, respectively^{39,40}.

Measurement of water quality

Dissolved oxygen (DO), water temperature (WT), pH, and conductivity (EC) in the influent and effluent of the device were measured by YSI meter (YSI ProQuatro, USA) on-site every four days. HACH turbidimeter (2100Q, HACH, USA) was used to measure the turbidity of water samples. Total nitrogen (TN), total phosphorus (TP), nitrate nitrogen ($\text{NO}_3^-\text{-N}$), nitrite nitrogen ($\text{NO}_2^-\text{-N}$), ammonia nitrogen ($\text{NH}_4^+\text{-N}$), phosphate ($\text{PO}_4^{3-}\text{-P}$) were determined by the national standards.

Characterization of *V. spinulosa* Yan growth

The leaves of *V. spinulosa* Yan were collected every 15 days to determine the chlorophyll concentration and enzyme activity of *V. spinulosa* Yan. Chlorophyll concentration was detected by ethanol

immersion method. The weighed leaves were first soaked in 95% ethanol for 24 h, then spectrophotometer was used to detect the soak solution at the wavelengths of 665 nm, 649 nm, and 470 nm to calculate the chlorophyll content. Activity of superoxide dismutase (SOD) and content of malondialdehyde (MDA) in the plant tissues were measured by kit method.

Analysis of microorganisms

At the end of the experiment, microbial samples were collected from the five areas in each environmental unit, including leaf surface, root surface, and root interior of *V. spinulosa* Yan, water, and sediment surface. The quality and quantity of DNA were examined by 1% agarose gel electrophoresis and NanoDrop2000, respectively. 16S rRNA (338 F, 5'-Actctacaggaggcagcag-3' and 806 R, 5'-Ggactacnvgggtwtctaat-3') V3-V4 variable regions were amplified by PCR. Sequencing was performed using Illumina's MiSeq PE300/NovaSeq PE250 platform (Shanghai Meiji Biomedical Technology Co., Ltd, China). USEARCH11 was used for sequence analysis. Sequences with $\geq 97\%$ of similarity were assigned to the same operational taxonomic unit (OTU). QIIME (v1.9.1) was used for analysis of sparse data set, including extraction and analysis of OTU, and annotation based on Bray-Curtis distance. All sequences could be obtained from NCBI SRA.

Data analysis

SPSS 26.0 software was used for statistical analysis of data. One-way analysis of variance was used to compare the difference between the experimental groups and the control groups, and the statistical significance level was 0.05. Kruskal–Wallis nonparametric test was used to analyze the data of individual parameters that did not conform to the normal distribution, determining the direct and indirect interactions of water characteristics (WT, DO, pH, and EC), water nutrients (TN, TP, $\text{PO}_4^{3-}\text{-P}$, $\text{NO}_3^-\text{-N}$, and $\text{NH}_4^+\text{-N}$), and large plant traits (Chlorophyll, biomass, SOD, and MDA). Microbial high-throughput data were analyzed and plotted using R 4.2.1 language. Effect size pipeline (LEfSe) program of the linear discriminant analysis (LDA) was used to test the significant discriminant groups under different stress conditions. In the LEfSe analysis, OTUs with LDA value > 3.0 were considered to be significant discriminant groups. The correlation between environmental factors, plant traits, and microbial indicators was analyzed by the R 4.2.1 method. In the correlation analysis, the first axis of the principal component analysis (PCA1) was used to represent the composition of the microbial community.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information file.

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Competing interests

The authors declare no competing interests.

Additional information

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