Research article

Substances released during the decomposition of *Vallisneria natans* and *Thalia dealbata*

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**HIGHLIGHTS**

- Different parts of aquatic plants had different rates of nutrient release.
- The DOM in the decomposed solution can be utilized by microorganisms.
- It is possible to use aquatic plant litter as an external carbon source.

**GRAPHICAL ABSTRACT**

- Water quality indicators
- Dissolved organic matter
- Fluorescence excitation emission matrices
- Parallel factor analysis

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**ABSTRACT**

Two types of aquatic plants commonly used for the ecological restoration of rivers and lakes, *Vallisneria natans* (Lour.) Hara and *Thalia dealbata* Fraser ex Roscoe, were selected and grouped by plant parts (root, stem and foliage), and decomposing release experiments were conducted. The influence of the released substances on the water quality was analyzed, as well as the amount of nutrients released by each part of these two plants. The calculated maximum chemical oxygen demand releases from the foliage of *V. natans* and the foliage of *T. dealbata* were approximately 5.4 g/kg and 22.65 g/kg, respectively. Through three-dimensional fluorescence spectrum and parallel factor analyses, the different material compositions of the decomposing liquids from the plants were determined, and the main dissolved organic components of the decomposing liquid of *V. natans* were amino-acid-like and microbially derived humics, and those of *T. dealbata* were soluble microbial by-product-like substances. The carbon-to-nitrogen ratio and humification index of each experimental group were compared. The experimental results showed that different parts of *V. natans* and *T. dealbata* had different rates of nutrient release. The dissolved organic matter in the decomposed solution can be utilized by microorganisms, which have the potential to become additional carbon sources. This study provides a new method for the treatment of aquatic plant litter. Different plant species can be used in combination according to their characteristics to ensure that better results are achieved during water treatment processes that use plant decomposing liquids as additional carbon sources.

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1. Introduction

With societal and economic development, water treatment in China has gradually developed from a focus on water quality standards to environmental ecological remediation. The current field of water treatment is not only focused on improving water quality; ecosystem restoration is also becoming increasingly important. During this process, constructed wetland technology has been widely researched and applied. Aquatic plants play an important role in constructed wetlands and have multiple functions. First, aquatic plants provide oxygen, carbon sources and attachment sites for microorganisms around their root systems (Tang et al., 2020), indirectly cultivating microorganisms in constructed wetlands and improving the efficiency of wastewater treatment. Second, aquatic plants can reduce the flow rate of wastewater and increase the sedimentation of solid waste (Kataki et al., 2021). Third, aquatic plants can absorb and store organic and inorganic substances in wastewater (Wang et al., 2021). Simultaneously, wetland plants have many other functions (Zhang et al., 2021), such as large-scale aquatic plants that can regulate the climate and landscape, and have landscape-formation functions.

Some studies have focused more on the collocation of wetland organisms and the effectiveness of pollutant removal from wastewater (Ji et al., 2020; Xu et al., 2020; Varma et al., 2021). The main mechanisms of pollutant removal in wetlands include biochemical transformation, adsorption, precipitation, volatilization, and plant uptake (Kataki et al., 2021). In addition to their beneficial side, contaminants that are absorbed or incorporated into plants are released back to the environment during the plant decomposition process when the plants have not been reaped, which is harmful because this poses the risk of secondary pollution in the ambient water. The decomposition of plants is a complex process that varies with the chemical properties of plant residues (Zhang et al., 2018), and the impacts of macrophyte decomposition on water quality is of concern. Some studies have shown significant differences in the decomposition products of different plants (Zhao et al., 2017). By managing the reaping of aquatic plants, the re-release of substances into the water environment can be avoided to a certain extent, which may stabilize the quality of the water environment (Sarkar et al., 2021). However, the disposal of the material after a plant has been reaped has become a new problem.

With regard to the disposal of plants litter, incineration of this plant litter (Tong et al., 2018), as well as resource utilization, including their use as fodder, herbs, biomass, energy and other positive uses were observed (Sarkar et al., 2021). Some studies have focused on the process of using plants litter as a carbon source, which may improve the nitrogen removal efficiency in constructed wetlands during the treatment process of low-carbon and high-nitrogen incoming water (Wu et al., 2018; Zhao et al., 2020). The decomposition of dissolved organic matter (DOM) in liquids is widely regarded as one of the primary drivers of microbial cycling and environmental changes in water quality. DOM is an important carrier of carbon and nutrients throughout ecosystems (Galloway et al., 2004; He et al., 2018). In addition, DOM in plant-decomposing liquids has been shown to play an important role in affecting water quality in aquatic ecosystems (Zhang et al., 2013). Compared with traditional carbon sources, additional carbon sources need to meet the characteristics of convenient material acquisition, low cost and environmental friendliness. The use of plant organic matter as an exogenous carbon source has become a popular research topic. Therefore, analyzing the composition of DOM in the decomposed solution of aquatic plants is useful in the analysis of these plants as carbon sources.

Currently, constructed wetlands are often used to further improve river water quality and for the deep purification of reclaimed water; however, incoming water is often inadequate in terms of being a carbon source, which results in inefficient nitrogen removal by the constructed wetland treatment process. Some researchers use local plant litter as an additional carbon source for constructed wetlands (Gu et al., 2021), but there are also some problems, such as the lack of differentiation between plant parts. The main reason for this is that the current aquatic plant reaping management is relatively inefficient. Traditional constructed wetland plants were usually reaped before winter, and the submerged plants of river-type wetland are frequently reaped for their overgrown parts. Aquatic plants are an important component of wetlands; however, most wetland plants are not fully utilized after being reaped. Because the water content of aquatic plants and the composition of various parts are different, it disposing of or reusing them indiscriminately is not conducive to improving their utilization efficiency. Simultaneously, if plant litter is not properly disposed of, water purification using wetland plants becomes meaningless. Therefore, selective and efficient recycling of the wetland plants may be a solution to this problem.

In this study, we analyzed the decomposition products of each plant part, such as roots, stems, and foliage. The effect of substance released during the decomposition of two aquatic plants on water quality was studied, and the composition of organic compounds in the decomposing solution was analyzed. The potential of these organic compounds as carbon sources was also investigated. The composition of DOM was measured using fluorescence excitation emission matrices. The humification index (HIX) and carbon-to-nitrogen ratio of each decomposition liquids were compared to analyze the possibility of using these litter types as external carbon sources. This study could guide the reaping management of common emergent and submerged plants, determine the appropriate parts of aquatic plants to be reaped, facilitate refined management, and improve the efficiency of plant utilization.

2. Material and methods

2.1. Raw materials

In this experiment, aquatic plants were reaped from the Jing River, which is a new reclaimed water recharge river in Beijing’s urban sub-center, with a water surface area of approximately 160,000 m² and a storage capacity of approximately 250,000 m³. The Jing River is 2.4 km long, and the normal water level of the river is 18 m at the elevation of the Yellow Sea. The Jing River is a compound section, with a 5 m wide shallow area on each bank, a water depth of 0.5 m, a gentle slope from the shallow area to the river bottom on both banks, and a slope ratio of 1:2.5. In the center of the river, the water depth is 1.8 m. The soil at the river bottom is clay, and the measured water flow after the completion of the river is 0.20 ± 0.05 m/s. The Jing River implemented ecological restoration and planted approximately 20,000 m² of aquatic plants in April 2019. Since then, the area covered by plants has gradually increased. In 2019, the annual mean values of plant cover in different areas of the Jing River ranged from 11.62% to 13.25%. Results from a 2020 plant survey indicated that the annual mean values of plant cover in different areas of the Jing River varied between 26.27% and 37.06%. Valliseria natans and Thalia dealbata were chosen because they are commonly used in ecological restoration.

The selected plant biomass was large and its regeneration ability was high. V. natans grew at the bottom of the water and T. dealbata grew in the shallow water area. Based on the above characteristics, the decomposition of both plants in water has an important impact on water quality. The large amount of organic matter released during decomposition has the potential to become a source of carbon for denitrification; therefore, these two plants were selected as experimental materials. By comparing the effects of substance release on water quality, different parts of the aquatic plants were sorted before the decomposition experiment.

Freshly collected aquatic plants were washed using tap water to remove sludge and other impurities, and then they were washed using ultrapure water several times to eliminate the interference of substances. The cleaned plants were sorted into different parts according to their physiological characteristics; V. natans was divided into roots and foliage (because this plant is a perennial stemless submerged macrophyte), while T. dealbata was divided into roots, stems and foliage. Finally, different parts of the aquatic plants were cut into sections approximately 2 cm long for subsequent use.
2.2. Experimental design

The experiments were conducted in a laboratory with good ventilation and light and environmental conditions as close to the natural environment as possible. The temperatures varied between 18 °C and 26 °C, and the wind speed was less than 1 m/s during the experiment. Sunlight shone normally into the lab location during the experiment, with sunrise being from 05:42 to 06:09 in the morning and sunset being from 18:46 to 17:59 in the evening. Using simulation based on the plant biomass in the real environment (Vymazal and Brezíňová, 2016), the raw materials were weighed, and 10 g of each plant part was taken from the chosen aquatic plants. The weighed samples were placed in a beaker filled with 3 L of distilled water, and these beakers were arranged in the experimental site for the decomposition experiments. Three sets of parallel experiments were conducted, as shown in Figure 1.

Sampling was conducted on days 0, 2, 4, 8, 12, 16, 20, 24, and 28, and water samples were collected to determine water quality indicators and DOM. The data measured on day 0 were the initial liquid conditions for each experimental group. During the experiment, 25 mL of water was collected from each vessel. After each sample test, an equal volume of distilled water was added to the experimental vessel. Although the distilled water would have a diluting effect on the decomposition solution, the analysis of its potential as a carbon source under this condition could adequately reflect the actual situation because the actual water environment is mostly continuous recharge. The results of the V. natans experimental group were stacked according to root and foliage, and the results of the T. dealbata experimental group were stacked according to root, stem, and foliage.

2.3. Analytical methods

Water quality indicators such as chemical oxygen demand (COD), total organic carbon (TOC), total nitrogen (TN), nitrate nitrogen (NO₃⁻–N) and total phosphorus (TP), were measured following standard methods (EPAC, 2002). During the experiment, the released concentrations of the substances in each part of the plant were plotted on bar graph, which was displayed in a stacked manner. The dissolved oxygen (DO) and pH values of each experimental set were determined in situ using a portable multi-parameter water quality analyzer (Proplus, YSI, USA). The carbon-producing capacities of the different plant decomposition solutions were compared by calculating the carbon-to-nitrogen ratio. The statistical significance of the difference between outcomes was calculated using pairwise t-tests.

During the early, intermediate, and late stages of the experiment, the DOM composition was measured using fluorescence excitation emission matrices (EEMs) and a fluorescence spectrophotometer (F-7000, Hitachi, Japan). Every nine days was a stage, and the sample collection time of each stage was the same as the water quality test time. The excitation wavelength was scanned from 200 nm to 650 nm, and the excitation interval was 5 nm. The emission wavelength was scanned from 200 nm to 650 nm, and the emission interval was 5 nm. The scanning speed was 12,000 nm/min and the voltage of the photomultiplier tube was 400 V.

Figure 1. Schematic diagram of the decomposition experiments: (A) foliage of Vallisneria natans, (B) root of V. natans, (C) foliage of Thalia dealbata, (D) stem of T. dealbata, (E) root of T. dealbata.
The decomposing liquids of the aquatic plants were filtered through a 0.25-μm water-based microporous membrane, injected into a special quartz cuvette and placed in a fluorescence spectrophotometer for scanning to obtain the fluorescence spectra of DOM in the decomposing liquids.

Based on the fluorescence of model compounds and DOM fractions, the EEMs were divided into five regions (Chen et al., 2003), and the DOM classification is presented in detail in Table 1.

Parallel factor analysis (PARAFAC) was used to decompose the fluorescence EEMs into their underlying chemical components (Murphy et al., 2013). PARAFAC is based on a mathematical model that represents the interactions between the dimensions in which the input data are to be analyzed (Schmitz et al., 2015). The model assumes that the fluorescence properties (fluorescence peaks) of each substance do not interfere with each other and that the model tool separates overlapping fluorescence peaks. PARAFAC was conducted using MATLAB (MathWorks, Natick, MA, USA) using the DOMFluor toolbox (Stedmon and Bro, 2008). The excitation and emission loadings of each component were compared with the OpenFluor database of PARAFAC aquatic fluorescence components to determine the type and source of the component (Juaporn et al., 2020).

During the decomposition of the aquatic plant litter, one of the fluorescence indices was calculated. The HIX, shown in Eq. (1), was used to evaluate the humification process (Tang et al., 2021).

\[
HIX = \frac{\sum_{Ex=254nm} I(Ex=254nm, Em)}{\sum_{Em=360} I(Ex=254nm, Em)} \tag{1}
\]

where I is the fluorescence intensity at each wavelength.

The HIX is a measure of the complexity and condensed (aromatic) nature of the DOM (Ohno et al., 2007). A high HIX value indicates more recalcitrant organics with higher aromaticity in the DOM. Conversely, the lower the HIX value, the easier it is to degrade and utilize and the more suitable it is for use as an external carbon source.

3. Results

3.1. Changes in water quality indicators

The COD concentration in each experimental group changed during the decomposition process. The COD released from the roots and foliage of V. natans were similar (Figure 2(A)), and the maximum release occurred on the 12th day, after which the concentration of COD decreased. The average concentrations of COD for each experimental group in the different parts (root and foliage) of V. natans increased from 8.0 mg/L to 25.0 ± 1.4 mg/L and 26.0 ± 4.2 mg/L, respectively.

The COD released from the roots, stems, and foliage of T. dealbata differed significantly, with the foliage experimental group having a higher COD release concentration than the other experimental groups (Figure 2(B)). Similarly, on day 12, the COD concentration of the foliage decomposed solution reached its highest (75.5 ± 10.6 mg/L). The other experimental groups may have been affected by the low concentration of COD; therefore, the overall change trend was not remarkable.

| EEM regions | Location of EEM peaks | DOM fractions          |
|-------------|-----------------------|------------------------|
| region I    | Ex < 250 nm, Em < 330 nm | aromatic protein       |
| region II   | Ex < 250 nm, 330 nm < Em < 380 nm | aromatic protein II |
| region III  | Ex < 250 nm, Em > 380 nm | fulvic acid-like substances |
| region IV   | Ex > 250 nm, Em < 380 nm | soluble microbial by-product-like substances |
| region V    | Ex > 250 nm, Em > 380 nm | humic acid-like substances |

EEM, excitation emission matrices; DOM, dissolved organic matter.
For the *T. dealbata* experimental groups, the concentration of TN released from different parts of the plant were differed remarkably. The TN concentrations in the experimental groups of the *T. dealbata* stem and foliage were higher than those in the experimental groups of the *T. dealbata* root. After the 12th day, the TN concentration in the foliage group was significantly higher than those in the other two groups, being stable at 5.50 ± 0.42 mg/L, and the stem and foliage groups also showed fluctuations of decreasing and then increasing (Figure 3(B)).

The TP concentration reached its highest value on the 20th day in both plant decomposition processes (Figure 4(A) and (B)). The average concentrations of TP for each experimental group in the different parts (root and foliage) of *V. natans* increased from 0.12 mg/L to 9.05 ± 1.48 mg/L and 11.65 ± 0.7 mg/L, respectively.

The concentrations of TP in the decomposition liquids of the root, stem, and foliage of *T. dealbata* differed. Furthermore, the foliage released the most TP during the decomposition process, with the highest TP concentration for the decomposition liquids being 6.95 ± 1.62 mg/L on the 20th day. At this time, the TP concentrations of the root and stem experimental groups were 0.55 ± 0.35 mg/L and 2.60 ± 0.28 mg/L, respectively.

### 3.2. Changes in the composition of dissolved organic matter

The results of the EEMs from the *V. natans* decomposition process (Figure 5(A–F)) showed that one of the fluorescence peaks for the root experimental group appeared at 230–240 nm/340–350 nm (Ex/Em) and another appeared at 270–280 nm/340–350 nm (Ex/Em). The peak locations (Ex/Em) were described in relevant regions, and these two peaks fell in regions II and IV, which represented aromatic proteins and soluble microbial by-product-like substances, respectively.

Four fluorescence peaks appeared during the early stages for the experimental foliage group. The two peaks that appeared differently were 230–240 nm/400–410 nm (Ex/Em) and 320–330 nm/400–410 nm (Ex/Em), which represented fulvic-acid-like substances (region III) and humic-acid-like substances (region V), respectively. During the intermediate and later stages of the experiment, these two substances disappeared from the EEMs.

The results of the EEMs from the *T. dealbata* decomposing process (Figure 6(A–I)) showed that, during the early stage of the experiment, the DOM concentration in the root experimental group was higher than that in the stem experimental group, and the DOM concentration in the foliage experimental group was the lowest. There were mainly three fluorescence peaks, and their positions were at 200–250 nm/300–350 nm (Ex/Em), 250–300 nm/300–350 nm (Ex/Em), and 350–400 nm/400–450 nm (Ex/Em) in regions II, IV, and V, representing aromatic proteins, soluble microbial by-product-like substances, and humic-acid-like substances, respectively.

As the experiment progressed, the fluorescence intensity of DOM in all experimental groups gradually decreased, and the amount of remaining DOM was small. From the comparative analysis of the fluorescence intensities, it was found that the concentration of DOM produced by the decomposition of *T. dealbata* was lower than that produced by the decomposition solution of *V. natans*.

### 3.3. Carbon to nitrogen ratio and humification index of plants litter decomposition solutions

The changes in the carbon-to-nitrogen ratio of each experimental group during the decomposition process were calculated (Figure 7); the carbon-to-nitrogen ratio of each experimental group was stable above 2 after 16 days, and on the 28th day, the carbon-to-nitrogen ratio was stable at approximately 4 for the roots of *T. dealbata* (T-root), stem of *T. dealbata* (T-stem), and root of *V. natans* (V-root). The other two experimental groups, that is, the foliage of *T. dealbata* (T-foliage) and the foliage of *V. natans* (V-foliage), had lower carbon-to-nitrogen ratios (ranging between 2 and 3).

The HIX was calculated to evaluate humification (Table 2). The HIX values of the *T. dealbata* experimental groups were generally higher than those of the *V. natans* experimental groups, and the foliage of the *T. dealbata* experimental group had the highest HIX value (0.310 ± 0.052).

### 4. Discussion

#### 4.1. Comparison of water quality indicators changes in plant decomposition solutions

The maximum concentration of COD could reach more than 51.0 mg/L if the root and foliage of *V. natans* decomposed in the same experiment. Certainly, changes in the experimental conditions may affect the microbial activity during the experiment, and, thus, affect the concentration of COD. Therefore, under the current experimental conditions, the calculated maximum COD releases from the root and foliage of *V. natans* were approximately 5.1 g/kg (fresh weight) and 5.4 g/kg (fresh weight), respectively. The maximum concentration of COD could reach more than 91.0 mg/L if the root, stem, and foliage of *T. dealbata* decomposed in the same experiment. The calculated maximum COD release from the foliage of *T. dealbata* was approximately 22.65 g/kg (fresh weight), and there were negligible COD emissions from the root and stem. In general, the COD release from *T. dealbata* was more than that from *V. natans*, and it was approximately four times higher than that of different parts of *V. natans* (such as the foliage).

The COD concentration of the decomposition solution of *V. natans* reached its highest value on the 12th day and then decreased (Figure 2). The COD concentration of the foliage experimental group was significantly higher than that of the other experimental groups in the *T. dealbata* decay experiment (Figure 51(A)). The reason for the change in the COD of the plant decomposition solution may be the change in DO conditions during the experiment (Figure S2) and microorganism decomposition (Zhao et al., 2017; Lu et al., 2018). The heterotrophic component of the experimental system was increased by eutrophication and organic matter decomposition from the plant litter. Decreases in DO caused by the prevalence of heterotrophy have been well-documented (Baxa et al.,...
The foliage experimental group was rapidly entering an anoxic state, while the other experimental groups were in the aerobic state, and some of the organic matter was decomposed.

The TN concentrations in each experimental group showed fluctuations, increasing and decreasing after the 4th day of the experiment. This may be due to microbial action (Asaeda and Nam, 2002; Galloway et al., 2004) and the adsorption–desorption of plant litter (Huang et al., 2020). Furthermore, the maximum concentration of TN may approach 6.00 mg/L if the root and foliage of *V. natans* were decomposed in the same experimental group. At the same time, the maximum release amounts of TN from the root and foliage of *V. natans* were 0.585 g/kg (fresh weight) and 0.825 g/kg (fresh weight), respectively. For the *T. dealbata*, the maximum concentration of TN may be above 10.00 mg/L if the root, stem, and foliage decomposed together. The maximum TN release from each part of *T. dealbata* was also calculated, in which the releases from root, stem, and foliage were 0.30 g/kg (fresh weight), 0.45 g/kg (fresh weight), and 1.83 g/kg (fresh weight), respectively. The amount of TN released by *T. dealbata* during the decomposition process was significantly higher than that released by *V. natans* (Figure S1(B)). Compared with foliage decomposition, the TN release from *T. dealbata* was approximately 2.2 times that from *V. natans*.

The TP concentration in each experimental group also showed fluctuations of increasing and decreasing. This may also be affected by microbial action and the adsorption–desorption of plant litter (Huang et al., 2020). The maximum releases of TP from the root and foliage of *V. natans* were 2.679 g/kg (fresh weight) and 3.459 g/kg (fresh weight), respectively. Moreover, the maximum release of TP in each experimental group (root, stem, and foliage) of *T. dealbata* was, and their values reached 0.129, 0.744, and 2.049 g/kg (fresh weight). Interestingly, we found that the amount of TP released by *V. natans* was higher than that released by *T. dealbata* (Figure S1(C)). This conclusion is contrary to those for COD and TN. This may be due to the different decomposition rates, decomposition patterns, and nutrient dynamics in aquatic ecosystems (Shilla et al., 2006). Therefore, it was normal for different plants to release different substances at different rates during the experiment.

During the experiment, there was no variability in the changes in DO and pH among the experimental groups, except for the differences between the DO changes in the foliage of the *T. dealbata* group and the other experimental groups (Figure S4).

### 4.2. Comparison of DOM changes in plant decomposition solution

DOM is a heterogeneous mixture of reduced carbon compounds and comprises the largest pool of active organic matter in aquatic environments (DeVilbiss et al., 2016). Two fluorescence peaks appeared in the roots of the *V. natans* experimental group, 230–240 nm/340–350 nm (Ex/Em) and 270–280 nm/340–350 nm (Ex/Em), which represented aromatic proteins and soluble microbial by-product-like substances. The fluorescence intensity represented the concentration of the substance, and the fluorescence peak was strongest during the intermediate stage of the root experimental group. Therefore, the concentration of DOM in the decomposing liquid of the *V. natans* root experiment group tended to increase first and then decrease. Proteins are major biochemical constituents of organisms and are thought to be susceptible to biological attacks. Soluble microbial by-product-like substances are protein-like substances metabolized by microorganisms. Protein-like fluorescence was detected almost ubiquitously in the samples. The amino acids phenylalanine, tyrosine, and tryptophan, all of which have aromatic rings, exhibit fluorescence properties (Yamashita and Tanoue, 2003). According to the 4-component model, PARAFAC analysis was performed to distinguish fluorescence EEMs into their underlying chemical components (Figure S5), and the component spectra were interpreted using the existing literature from the OpenFluor database (Murphy et al., 2013), with a minimum similarity score (congruence coefficient) of 0.90.
Component C1 corresponds to amino-acid-like (Kim et al., 2020), and C3 corresponds to microbially derived humics (Philibert et al., 2022).

In the foliage of the V. natans experimental group, there were four fluorescence peaks, of which two were similar to the root experimental group and the other two appeared differently at 230–240 nm/400–410 nm (Ex/Em) and 320–330 nm/400–410 nm (Ex/Em), respectively, which represented fulvic-acid-like substances and humic-acid-like substances. Fulvic-acid-like substances have a single excitation wavelength and contain a large number of phenolic hydroxyl, carbonyl, and other functional groups. Humic-acid-like compounds represented fluorescence components with maximum excitation and emission wavelengths, the widest excitation and emission bands, and their aromaticity was greater than that of the fulvic-acid-like compounds (Zhang et al., 2020). Excitation and emission loading of the four PARAFAC-derived components of DOM obtained during the decomposition of the foliage of V. natans are shown in Figure S6. Component C2 corresponded to microbially derived humic-like components (Dainard et al., 2015).

The soluble microbial by-product-like substances showed the ‘protein-like’ or ‘amino-acid-like’ fluorescence, as derived from tyrosine, tryptophan, or protein in the DOM. The results showed that the main dissolved organic components of the decomposing liquid of V. natans were easily utilized by microorganisms.

For the T. dealbata decomposition processes, based on the fluorescence intensity, the DOM concentration in the root, stem, and foliage experimental groups gradually decreased.

PARAFAC analysis was performed to distinguish fluorescence EEMs into their underlying chemical components for the T. dealbata experimental groups. According to the 4-component model, the obtained components were mainly soluble microbial by-product-like substances (microbially derived humic-like substances) (Figures S7, S8, and S9). Component C2 corresponded to microbially derived humic-like components (Dainard et al., 2015). The difference was that, in the foliage experimental group of T. dealbata (Figure S9), humic-acid-like substances appeared. Components C3 and C4 corresponded to humic-like components (Stedmon and Markager, 2005; Dainard et al., 2019). This showed
that the structure of dissolved organic matter in the foliage experimental group of *T. dealbata* was more complex, and it was more difficult for microorganisms to utilize.

### 4.3. Potential analysis of aquatic plant decomposing liquid as carbon source

To further analyze the possibility of using the two selected aquatic plant decomposition products as carbon sources, analyses was performed using the carbon-to-nitrogen ratio and HIX. The carbon-to-nitrogen ratios of the decomposing liquid in each part of the aquatic plants differed. When the carbon-to-nitrogen ratio of the water was lower than 2, it was not conducive to the denitrification of microorganisms, and this type of water required other intensive treatments (Deng et al., 2015). The HIX increased due to the large input of aromatic and humic DOM, which means that a higher value of HIX indicates more recalcitrant organics with higher aromaticity in the DOM (Lee et al., 2019), suggesting that the DOM was more difficult to utilize by microorganisms.

The HIX provides a measure of the DOM humification status (Inamdar et al., 2012). There were no significant differences in the HIX values between the experimental groups, except for the foliage of the *T. dealbata* and *V. natans* experimental groups (Figure S12). The decomposing liquid of the *V. natans* groups had a lower HIX than that of the *T. dealbata* groups. The results indicated that the decomposing liquid of *V. natans* was more easily utilized by microorganisms and relatively more suitable for use as an external carbon source. Considering that the root and foliage of *V. natans* release similar amounts of other substances, during the actual plant reaping management process, plant maintenance personnel should only reap the foliage part of *V. natans* to remove more nutrients and then use them as external carbon sources. For the management of *T. dealbata*, the stem and foliage parts should be separated for classification and utilization.

### Table 2. Humification index values for each experiment.

| Experiment group       | The value of HIX      |
|------------------------|-----------------------|
| The root of *Vallisneria natans* | 0.132 ± 0.084         |
| The foliage of *Vallisneria natans* | 0.138 ± 0.070         |
| The root of *Thalia dealbata* | 0.222 ± 0.136         |
| The stem of *Thalia dealbata* | 0.191 ± 0.136         |
| The foliage of *Thalia dealbata* | 0.310 ± 0.052         |

5. Conclusions

Under the current experimental conditions, the calculated maximum COD releases from the root and foliage of *V. natans* were approximately 5.1 g/kg and 5.4 g/kg, respectively. The calculated maximum COD release from the foliage of *T. dealbata* was approximately 22.65 g/kg, and there were negligible COD emissions from the root and stem of *T. dealbata*. The amount of TN released by *T. dealbata* during the decomposition process was higher than that released by *V. natans*, and the maximum release amounts all appeared in the foliage experimental groups, were 1.83 g/kg and 0.825 g/kg for *T. dealbata* and *V. natans*, respectively. The amounts of TP released by *V. natans* (root and foliage, 2.679 g/kg and 3.459 g/kg, respectively) were higher than those released by *T. dealbata* (root, stem, and foliage: 0.129 g/kg, 0.744 g/kg, and 2.049 g/kg, respectively).

According to the PARAFAC analysis, the main dissolved organic components of the decomposing liquid of *V. natans* were amino-acid-like and microbially derived humics, and the main dissolved organic components of the decomposing liquid of *T. dealbata* were soluble microbial by-product-like substances (microbially derived humic-like substances), which can be easily utilized by microorganisms. The HIX values of the *V. natans* experimental groups were generally lower than those of the *T. dealbata* experimental groups; the lower the HIX value, the easier it was to be degraded and utilized. Given that the carbon-to-nitrogen ratio and HIX value of the decomposing liquid in each part of the aquatic plant differed, the different plant parts could be used together in applications, and the carbon-to-nitrogen ratio and HIX value could be adjusted to more suitable values. During the actual plant reaping management process, for the management of *V. natans*, plant maintenance personnel should only reap the foliage to remove more nutrients and then use them as external carbon sources. For the management of *T. dealbata*, the stem and foliage parts should be separated for classification and utilization.

As the research data were derived in the laboratory, there are still limitations. In the actual environment, the characteristics of incoming water quality, microbial activities, and changes in environmental factors are complex and variable. Therefore, in the future, similar studies should be conducted in actual constructed wetlands to further evaluate the actual potential of plant litter as carbon sources.

### Declarations

**Author contribution statement**

Zhaoxin Li: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Peng Liu: Performed the experiments; Analyzed and interpreted the data.

Zhiyan Sun: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ning Ma: Contributed reagents, materials, analysis tools or data.

Jijian Lian: Contributed reagents, materials, analysis tools or data.

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