Influent salinity affects substrate selection in surface flow constructed wetlands

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Abstract

Conducted in pilot-scale surface flow constructed wetlands (SFCWs), this study's objective is to identify the effect of influent salinity on substrate selection. Compared with gravel and sand SFCWs, soil SFCWs performed similarly to worse and similarly to better at low and high salinities, respectively, in removal efficiencies (REs) of salt, total nitrogen (TN), total phosphorous (TP) and chemical oxygen demand (COD). Soil generally increased macrophyte growth (especially at high salinity) in dry biomass, leaf chlorophyll concentration, root activity and root catalase and superoxide dismutase activities. The decrease of bacterial diversity in rhizosphere may be caused by high salinity, while soil improved rhizosphere bacterial community stability at varying salinities more than gravel or sand. At high salinity, soil support of macrophytes and rhizosphere microorganism increased pollutant REs in SFCWs or at least offset relatively poor biofilm attachment to soil. This highlights the necessity of the varying substrate selection in SFCWs with influent salinities for both increasing pollutant REs and reducing input cost.

1. Introduction

In constructed wetlands (CWs), especially in surface flow CWs (SFCWs) with relatively low purification function (PF) per surface unit when compared with subsurface flow CWs (SSFCWs) filled with large amounts of natural or artificial external substrates (ESs) (such as gravel, sand and ceramsite), the role of macrophytes should not be neglected because macrophytes can improve the PF of CWs by direct absorption, adsorption, sedimentation, degradation of root biofilm and improvement of the rhizosphere environment through root radial oxygen loss (ROL) and organic matter secretion (Sun et al., 2019; Zhao et al., 2018a). The promotion of macrophyte growth has special significance for the PF of SFCWs (Klomjek & Nitorisavut, 2005; Li et al., 2020).

To improve the PF and mitigate clogging of wetlands, ES was also recommended to replace or partially substitute for the native soil for SFCWs or the top layers of subsurface/vertical flow CWs to increase the absorption and attached biofilm degradation of the substrates (Iamchaturapatr et al., 2007; Zheng et al., 2016; Zheng et al., 2014). This design idea has been widely used in the improvement of natural wetlands and the construction of CWs (Wang et al., 2018). However, compared with ESs such as gravel, sand and slag, native soil/silt benefits the growth of aquatic macrophytes and thus has the potential to improve the PF of wetlands (Li et al., 2012). Especially under environmental stresses such as high/low pH, high salt and toxic chemicals, soil can protect roots and stimulate the growth of macrophytes by mechanisms such as buffer action and exchange adsorption of soil colloids. Therefore, in the selection of substrates for SFCWs, both the purification ability of the substrate and the influence of the substrate on macrophyte growth, which probably vary with environmental stresses, should be considered. Currently, there are few studies on the influence of environmental stress on the selection of substrates for CWs.

Salt stress is a common challenge for CWs treating wastewater from industrial and agricultural production with seawater as the water source and high-salinity wastewater from industrial production. Under salt stress, both macrophyte growth and microbial activity are greatly affected, and thus, the PF of

CWs generally significantly decreases (MacTavish & Cohen, 2017; Sheng et al., 2015). On the one hand, soil can supply better nutrient and rhizosphere environments for the growth of macrophytes than the substrates sand, gravel and ceramsite and thus improve the resistance to salt stress, which has specific significance for SFCWs; In addition, soil can affect the PF of wetlands by altering the water salt concentration by deposition, resuspension and exchange adsorption of sediment colloids. Therefore, the performances of the native soil and ES in the PF of wetlands probably varied with salt stress, which is still a research gap in the CW community. The related research can supply a valuable reference for the construction of CWs, which is beneficial to not only increasing the PF of CWs but also reducing the construction cost under the background of price increase for ES such as gravels and sands by the control of the mining for the ecological security and restoration (Zhai et al., 2020).

As a result, with widely used materials (i.e., the native soil and the ES of sand and gravel) as substrates, the overall objective of this study is to identify soil application strategies in SFCWs under varying salt stress by comparing its performance with sand and gravel applications. There are three specific sub-objectives in addressing this overall objective: (1) identifying the effect of salt stress on the REs of salt, TN, TP and COD of SFCWs with soil, sand and gravel as substrates and (2) comparing the status of macrophytes and rhizosphere bacteria under different substrates and varying salt stress and (3) identifying the effects of the soil supporting macrophytes and rhizosphere bacteria on the PF of SFCWs under varying salt stress.

2 Materials And Methods

2.1 Experimental site and design

From May to July in 2019, Pilot-scale SFCW systems was officially carried out at Hongze Wetland Experimental Station of Nanjing University (118°55'27" E, 33°20'13" N) (Zhao et al., 2020) in fiber-reinforced plastic rectangular incubators with dimensions of 1.10*0.81*0.44 m (length*width*height). The Temperature and Illuminance Data Logger (HOBO Pendant UA-002-08, Onset, USA) was used to record the real-time changes of water temperature. In the course of the experiment, the daily average water temperature was 23.74 °C and fluctuated between 20.65 °C and 27.92 °C in the experimental period (Supplementary information). A widely used macrophyte species in CWs, yellow iris (*Iris pseudacorus*), was selected due to its relatively high salt tolerance (Lu & Peng, 2012).

The experiment included three treatments with different SFCW substrates, i.e., the native soil, sand (1–2 mm in diameter) and gravel (10–20 mm in diameter, porosity of 0.45), which are termed SO, SA and GR in the following text, respectively. The chemical property of the substrates are shown in Table 1. For the convenience of macrophyte sampling, seedlings of plants of similar size were planted in plastic flowerpots (cylinders with diameters and heights of 16.0 cm and 13.8 cm, respectively). A total of 24 flowerpots were closely placed in the incubators of SFCWs. The layers of substrates, i.e., soil, sand or gravel, were 12.5 cm deep. Three duplicates were included, and a total of nine SFCW systems were established (Supplementary information). Before the experiment, the macrophytes were precultured using

water from a nearby pond for 15 days with relatively low concentrations of COD, TN, TP and salt (Zhao et al., 2018b). The water quality data indicators of each period are shown in Table 2.

Table 1

The chemical characteristics of the substrates used in the surface flow constructed wetlands

Substrate	Organic matter (g·kg ⁻¹)	Total nitrogen (mg·kg ⁻¹)	Available phosphorous (mg·kg ⁻¹)	Available potassium (mg·kg ⁻¹)	Cation exchange capacity (cmol·kg ⁻¹)
Soil	9.40	684	8.30	148	7.82
Sand	0.83	ND	2.0	17	4.50
Gravel	CD	CD	CD	CD	CD
Note: ND = not detected; CD = cannot be detected.					

2.2 System operation

The experiment was conducted in continuous water flow mode, and the influent came from a water tank of 5.0 ton. To stabilize the water flow and reduce the water flow deviation among the nine SFCW systems, the water in the tank was pumped out through a reinforced plastic pipe using a booster pump, the flow was divided into three flows by a triage valve, and each flow was divided into more three flows. The experiment was started on May 14, 2019 and each period is 15 days, a total of four periods. In the four periods, the influent salinities were 0.8‰, 1.5‰, 3.0‰ and 6.0‰, respectively. Synthetic wastewater was composed composed of the pond water nearby and the secondary wastewater from the sewage-treatment plant nearby. The influent characteristics of the four periods are shown in Table 2. The hydraulic retention time was 2 days, and the water level remained at 22 cm throughout the experiment.

Table 2

The influent water characteristics in the four periods

Period	Source	Salinity ‰	COD (mg·L ⁻¹)	NH ₄ ⁺ -N (mg·L ⁻¹)	TN (mg·L ⁻¹)	TP (mg·L ⁻¹)
I	50% pond water + 50% secondary water	0.8	45	2.10	7.5	0.65
II	100% secondary water	1.5	50	4.40	15.1	1.35
III	100% secondary water + salt	3.0	55	4.75	16.35	1.79
IV	100% secondary water + salt	6.0	56	4.26	16.5	1.47

2.3 Sampling and analysis

Sampling is arranged at the end of each period, including water, plants and substrates. According to the standard analysis procedure, the concentrations of COD, TN and TP were measured by water quality analyzer (drb200 and DR2800, HACH, USA) (Zhao et al., 2018b). The water salinity was measured using a salinometer (Sanxin sx-650, Shanghai, China). A chlorophyll meter, SPAD 502 (Single-Photon Avalanche Diode, Konica Minolta, Japan) was used to measure leaf chlorophyll concentration. Each time, two samples of plants were taken: one was used to measure aboveground and belowground biomass, which were dried at 105 °C for 15 minutes and 75 °C to constant weight; the other was used to measure the plant physiological parameters including root activity, root CAT activity and root SOD activity. Triphenyltetrazolium chloride (TTC) method (Bai et al., 1994) was used to measure root activity. The determination of CAT activity of root was determined by the ultraviolet absorption method (Romero-Oliva et al., 2015) and the determination of SOD activity of root was determined by photochemical reduction of nitrogen blue tetrazole (NBT) (Wang & Huang, 2014).

Rhizosphere microorganism samples were sent to Majorbio-pharmaceutical Technology company(Ltd.; Shanghai, China) for determination, Qiime online analysis system (version 1.8.0, <http://www.majorbio.com/>) was used to calculate rhizosphere bacterial community structure indices, observed species, the Shannon index and Venn analysis..

2.4 Data processing

The data were analyzed by SPSS 2.0 (IBM,USA). The effect of treatment according to the pollutant RE, plant physiological and ecological indexes and rhizosphere bacterial community was compared by One-way analysis of variance (ANOVA) ,The correlation among the pollutant RE, plant physiological and ecological indexes and rhizosphere bacterial community was performed by using redundancy analysis (RDA) (Canoco 5.0).

3. Results And Discussion

3.1 Pollutant removal efficiency

At 0.8‰ salinity, all three treatments showed negative salt REs (Fig. 1A). With increasing salinity, salt RE increased first and then slightly decreased for treatments SA and GR, while for treatment SO, salt RE gradually increased. Treatment GR consistently showed the significantly lowest salt RE at all the test salinities, with p values ranging from 0.005 to 0.044. Compared with SA, SO showed similar or slightly lower salt RE at salinities below 3.0‰, while at 6.0‰ salinity, SO had a significantly higher salt RE (p = 0.037).

At low salinity (0.8‰), no significantly different REs were obtained between the three treatments for either TN or TP (Fig. 1B and 1C). At salinities of 1.5‰ and 3.0‰, GR showed the highest TN and TP REs, while compared with SA, SO showed significantly lower REs of TN (p = 0.042) and TP (p = 0.032) at 1.5‰ salinity and no significantly different REs of TN (p = 0.891) and TP (p = 0.181) at 3.0‰ salinity. At 6.0‰

salinity, no significantly different TN REs were observed between the three treatments; SO showed the highest TP RE of the three treatments.

With increasing influent salinity, COD RE increased first and then decreased for all three treatments, with peaks at 1.5‰ salinity (Fig. 1D). At 0.8‰ salinity, no significant difference in COD RE was observed between treatments. At 1.5‰ salinity, GR and SO showed the highest and lowest COD REs, respectively. At salinities of 3.0‰ and 6.0‰, SO had the highest COD RE, while GR had the lowest COD RE.

The results suggested that influent salinity greatly influenced the performance of the SFCWs with different substrates. Generally, at low salinity, the soil SFCWs generally performed worse or similarly to the sand or gravel SFCWs in the REs of salt, TN, TP and COD, while at high salinity, the soil SFCWs generally performed better or similarly to the sand or gravel SFCWs. Therefore, at high influent salinity, native soil was recommended as the substrate of SFCWs.

The influence of influent salinity on the performance variation between the SFCWs can be explained by the direct and indirect effects of the substrates. The direct effects of substrates include the supplying of attachment surface for biofilms and the adsorption of pollutants (Wang et al., 2020; Yan et al., 2017; Yang et al., 2018; Zou et al., 2016). The indirect effects of substrates mainly come from their influence on macrophytes, which can affect the PF of SFCWs by direct absorption, supplying a biofilm attachment surface, functioning as a carbon source and ROL (Mei et al., 2014; Zhao et al., 2018a; Zhao et al., 2018b). Compared with sand and gravel, soil probably has stronger colloid adsorption and a lower ability to supply an attachment surface to biofilms due to the limited water flow between soil particles (Wang et al., 2020; Yan et al., 2017). However, compared with sand and gravel, soil can probably benefit the growth of macrophytes by nutrient accumulation in soil (Jiang et al., 2008; Zhu et al., 2019).

3.2 Macrophyte growth

Biomass, including aboveground biomass and belowground biomass, is regarded as the key characteristic of macrophytes suitable for CW design (Vymazal, 2011). With increasing influent salinity, both the aboveground and belowground biomass gradually increased (Fig. 2A), which suggested that the macrophyte species *Iris pseudacorus* had relatively high salt tolerance. At 0.8‰ salinity, there was no significant difference in aboveground biomass between the three treatments, while treatments of SO and SA had significantly higher belowground biomass than GR, with p values of 0.008 and 0.005, respectively. Significantly higher aboveground and belowground biomass was observed in SO than in either SA or GR when the influent salinity was higher than 1.5‰, with p values ranging from 0.002 to 0.048.

The SPAD value, a parameter quantifying leaf chlorophyll concentration, increased first and then slightly decreased when influent salinity increased from 0.8 to 6.0‰ for treatment SO (Fig. 2D), while for treatments SA and GR, general decreasing trends were observed. At salinities higher than 1.5‰, SO showed significantly higher SPAD values than SA and GR, with p values ranging from 0.01 to 0.044. Root activity generally decreased with increasing salinity (Fig. 2E). With increasing salinity, treatment SO decreased slower than SA and GR. Compared with the preculture period, root activity had a 52.29% decrease for SO, while SA and GR root activities decreased 66.51% and 72.77%, respectively.

Root CAT activity, indicating the ability to resist oxidative stress, generally increased first and then showed decreasing trends with increasing salinity (Fig. 2B). Root SOD, another antioxidant enzyme, gradually increased or increased first and then slightly decreased (Fig. 2C). Treatment SO showed the highest CAT activity and SOD activity at the high salinities of 3.0‰ and 6.0‰, while at salinities of 0.8‰ and 1.5‰, SO showed relatively lower or similar CAT activity and SOD activity than SA and GR.

Similar to the pollutant RE results, the above results suggested that influent salinity greatly influences the performance variation between SFCWs with different substrates. Generally, compared with sand and gravel SFCWs, the native soil SFCWs showed no clear superiority in stimulating macrophyte growth at low influent salinity, which varied with the evaluation parameters, while at high influent salinity, the native soil SFCWs generally performed better than the sand or gravel SFCWs in all the above parameters, i.e., aboveground biomass, belowground biomass, SPAD value, root activity, and CAT and SOD activities. Therefore, compared with the ESs sand and gravel, native soil can mainly benefit macrophyte growth under high salt stress.

There are probably several pathways for the stimulation of macrophyte growth by soil compared with that of sand or gravel. First, improving the nutrient supply by soil enrichment has special significance for macrophyte growth. Compared with widely used plant total nutrient solutions, such as Hoagland solution, the nutrient concentrations in most eutrophic water, including the influent water in this study, are much lower than the demanded concentrations, which is probably the main reason why soil instead of water is the main source of nutrients for most rooted macrophytes (Jiang et al., 2008). Therefore, the nutrients enriched in soil by particle deposition, exchange adsorption of soil colloids and biological activity can greatly stimulate macrophyte growth and improve macrophyte resistance (Tadros et al., 2019; Zhao et al., 2018a; Zou et al., 2016). Second, compared with sand and gravel, soil can increase rhizosphere microbial abundance and thus benefit macrophyte growth because rhizosphere microorganisms stimulate the growth of macrophytes by providing nutrients and plant hormone secretion (Marschner et al., 2001; Qin et al., 2019; Vergani et al., 2017; Xu et al., 2009). Third, the soil buffering function and limited water flow in soil can protect macrophyte roots by decreasing salt stress. The large exchangeable ion adsorption capacity of soil colloids, compared with the capacities of sand and gravel, can decrease the water salinity and thus the salt stress (Wang & Wang, 2019), which has been proven by this study; soil having much smaller particles can limit the water flow between particles and thus protect the roots by decreasing the contact of the water salt and root (Wang et al., 2020).

3.3 Rhizosphere bacteria

3.3.1. Bacterial diversity

The α diversity of rhizosphere bacteria was analyzed using the Shannon index and observed species number (Fig. 3). With increasing influent salinity, the Shannon index for the three treatments gradually decreased or decreased first and then slightly increased. At the four salinities, the Shannon indexes of treatments SO, SA and GR fluctuated in the ranges 5.53–6.79, 3.04–6.16 and 3.76–5.94, respectively, which suggested that, compared with sand and gravel, soil could make the α diversity of rhizosphere

bacteria relatively stable with the variation in salinity. For the observed species number, a similar result was obtained: for SO, the species number gradually decreased from 2840 to 2098, while for SA and GR, the species number fluctuated from 1302–2917 and from 823–2041, respectively. The results suggested that increasing salinity could generally decrease rhizosphere bacterial α diversity and that when compared with SA and GR, SO could make α diversity relatively stable.

3.3.2. Bacterial community structure

The dominant rhizosphere bacteria at both the phylum and genus levels are listed in Fig. 4. Generally, the results suggested that bacterial community structure varied largely with influent salinity and substrate, including functional bacteria involved in the transformation of nitrogen, phosphorous and COD (Li et al., 2019b; Li et al., 2020) and bacteria related to plant growth (Wang et al., 2019). However, halophilic bacteria such as genera *Halobacteriovorax* and *Halomonas* and the unclassified family *Halobacteroidaceae*, distributed widely in saline soil (Li et al., 2019a), were not included in the genera with a relative abundance higher than 0.01%, which was probably related to the relatively low influent salinity.

Among the SO, SA and GR treatments, the same bacterial numbers at the genus level were 663, 577, 553 and 505 for influent salinities of 0.8‰, 1.5‰, 3.0‰ and 6.0‰, respectively, accounting for 53.3%, 53.3%, 43.8% and 41.3% of the total number of genera, respectively (Supplementary information). This result suggested that increasing salinity could stimulate variation in the bacterial community between treatments.

Among the four salinities, Venn diagrams suggested that the same bacterial numbers at the genus level were 656, 608 and 491 for the SO, SA and GR treatments, respectively, accounting for 56.6%, 53.0%, and 47.6% of the total number of genera, respectively (Supplementary information). This result suggested that compared with the substrates sand and gravel, the native soil could make the community structure of rhizosphere bacteria more stable, which is consistent with the results of this study for bacterial diversity.

The facilitation of salinity tolerance of macrophytes by soil is probably how soil stabilizes the rhizosphere bacterial community under varying salinities. Numerous studies have suggested that rhizosphere bacteria and plant growth significantly interact: the rhizosphere microbial community promotes plant health, productivity and stress resistance by a series of direct and indirect mechanisms, such as the use of plant growth-promoting bacteria (PGPB) (Berendsen et al., 2012), while plant growth can benefit microbes by the release of carbon dioxide and secretion of photosynthate (Ahkami et al., 2017; Singh et al., 2018). Compared with gravel and sand, soil improved the salinity tolerance of macrophytes under high salinities (Fig. 2), which could benefit the development or stability of the rhizosphere microbial community and thus create a virtuous cycle between microbes and macrophytes (Radhakrishnan & Baek, 2017).

3.4 The effects of macrophytes and rhizosphere bacterial communities on pollutant RE

The relationship between the macrophyte growth parameters, rhizosphere bacterial diversity indices and pollutant RE was analyzed using the RDA method (Fig. 5). A correlation significance test suggested that

pollutant REs generally had no significant correlations with the macrophyte parameters and the rhizosphere bacterial diversity at a salinity of 0.8‰ (Table 3). This could be explained by the offsetting effects of the increasing and decreasing pathways for RE in soil when compared with sand and gravel, i.e., the increases by the stimulation of macrophyte growth in biomass and the decreases by the biofilm attachment function (Wang et al., 2020). Generally, compared with macrophytes, substrates are the dominant factor determining pollutant RE ability (Mateus & Pinho, 2020; Yang et al., 2018). However, at a salinity of 0.8‰, the advantage of sand or gravel to soil in biofilm attachment supply was probably relatively limited in the startup period of the CWs (Bo et al., 2015; Ramond et al., 2012) and resulted in the canceling effect.

Table 3

The correlation significance test for the pollutant RE with macrophyte physiological and ecological indexes and rhizosphere bacterial parameters at influent salinities of 0.8‰, 1.5‰, 3.0‰ and 6.0‰ (the abbreviations are the same as those in Fig. 5).

Salinity	Variable	AB	UB	RA	CAT	SOD	SPAD	S _{obs}	Shannon
0.8‰	SALT	X	*	X	X	X	X	*	X
	TN	X	X	X	X	X	X	X	X
	TP	X	X	X	X	X	X	X	X
	COD	X	X	X	X	X	X	X	X
1.5‰	SALT	X	X	X	*	*	X	X	X
	TN	X	X	- *	X	X	X	X	X
	TP	- **	- *	X	X	X	X	X	X
	COD	X	X	X	X	X	X	X	X
3.0‰	SALT	X	*	X	X	*	X	*	X
	TN	X	- **	- **	X	- **	X	- **	- *
	TP	X	- **	- *	X	- **	X	- *	X
	COD	*	X	X	X	X	**	X	*
6.0‰	SALT	*	**	**	*	**	**	*	*
	TN	X	X	X	X	X	X	X	X
	TP	X	X	X	X	X	X	X	X
	COD	X	*	**	*	**	**	X	*

Note: X = Not significant, * = significant at p = 0.05, ** = significant at p = 0.01.

With increasing salinity and the development of biofilm attached on the substrate surface, the advantages of sand or gravel gradually overwhelmed the stimulation of macrophyte growth by soil for REs of TN and TP, and thus REs of TN and TP showed significantly negative correlations with some macrophyte and rhizosphere bacterial parameters at salinities of 1.5‰ and 3.0‰. Microbial activity was on one hand inhibited by the high salinity at 6.0‰ (MacTavish & Cohen, 2017; Sheng et al., 2015), and thus, the advantage of sand or gravel for biofilm attachment probably decreased; on the other hand, the advantages of soil in stimulating macrophyte growth and rhizosphere bacterial community stability probably increased (Jiang et al., 2008). Thus, the positive and negative effects of soil on the REs of TN and TP again canceled, which resulted in the no significant correlations of TN and TP REs with macrophyte and rhizosphere bacterial parameters.

Meanwhile, the REs of COD and salt gradually positively significantly correlated with an increasing number of parameters of macrophytes and rhizosphere bacteria with increasing salinity. For salt RE, this temporal dynamic was probably due to the increasing stimulation of macrophytes and rhizosphere bacteria by soil compared with the effects of sand and gravel and the increasing exchange adsorption of soil colloid (Yan et al., 2017), while for COD RE, this phenomenon was probably also related to increasing withering of macrophytes and COD release from the decomposition of litter for the sand and gravel CWs over the those of the soil CWs (Zhao et al., 2018a).

Therefore, at low salinities (< 3.0‰) other than 0.8‰ in the startup period of the CWs, compared with sand and gravel SFCWs, soil SFCWs exhibited similar or lower TN and TP REs due to the role by the substrate in biofilm attachment being more overwhelming than the facilitation of macrophyte growth, while at high salt stress (i.e., 6.0‰ salinity), soil exhibited similar TN and TP REs as sand or gravel due to the offsetting negative and positive effects. For REs of salt and COD, with increasing salinity, soil performed increasingly better than sand and gravel due to its support of macrophytes and rhizosphere bacteria. Therefore, it is recommended to use soil in SFCWs or even in SSFCWs as the top layer at high influent salinity or large fluctuation of salinity, in order to not only increase the stability of PF but also decrease input cost of CW establishment due to the control of sand and gravel mining (Zhai et al., 2020).

4. Conclusions

Soil could facilitate macrophyte growth and rhizosphere bacterial community stability, especially at high salinity, which can either improve pollutant REs or maintain a similar performance, at least by offsetting the relatively low biofilm attachment of soil when compared with those of sand and gravel. Therefore, it is recommended to use soil as the substrate of SFCWs at high salinity or large fluctuation of salinity to facilitate macrophyte growth and stabilize rhizosphere microorganisms and thus improve PF. Besides of the improved stability of PF in SFCWs, this strategy can greatly decrease cost of CW establishment due to the easier availability of soil when compared with sand or gravel.

Declarations

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Ethical Approval and Consent to Participate

Not applicable

Consent to Publish

Written informed consent for publication was obtained from all participants.

Authors Contributions

Shenyan Zhou: methodology, validation, data curation, investigation, writing—original draft, resources. Ran Zhao: validation, investigation. Qiming Li: validation, investigation. Juan Du: validation, data curation, investigation. Chen Chen: investigation, resources. Qianqian Lu: resources, investigation. Miao Zhang: data curation, investigation, resources. Dehua Zhao: conceptualization, data curation, writing—review and editing, supervision. Shuqing An: writing—review and editing, funding acquisition.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

The data sets supporting the results of this article are included within the article and its additional files.

References

Ahkami, A.H., Allen White, R., Handakumbura, P.P., Jansson, C., 2017. Rhizosphere engineering: Enhancing sustainable plant ecosystem productivity. *Rhizosphere* 3, 233-243.

- Bai, B., Jin, J., Bai, S., Huang, L., 1994. Improved method for determination of TTC in maize root system. *Corn Sci.* 2, 44-47.
- Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., 2012. The rhizosphere microbiome and plant health. *Trends Plant Sci.* 17, 478-486.
- Bo, Y., Yu, I., Chen, T., Fan, P., Jin, K., 2015. Biofilm formation and start-up of denitrification subsurface constructed wetland. *Chin. Wat. Wastewat.* 31, 10-13.
- Iamchaturapatr, J., Yi, S.W., Rhee, J.S., 2007. Nutrient removals by 21 aquatic plants for vertical free surface-flow (VFS) constructed wetland. *Ecol. Eng.* 29, 287-293.
- Jiang, J., Zhou, C., An, S., Yang, H., Guan, B., Cai, Y., 2008. Sediment type, population density and their combined effect greatly change the short-time growth of two common submerged macrophytes. *Ecol. Eng.* 34, 79-90.
- Klomjek, P., Nitisoravut, S., 2005. Constructed treatment wetland: a study of eight plant species under saline conditions. *Chemosphere* 58, 585-593.
- Li, H., Chi, Z., Li, J., Wu, H., Yan, B., 2019a. Bacterial community structure and function in soils from tidal freshwater wetlands in a Chinese delta: Potential impacts of salinity and nutrient. *Sci. Total Environ.* 696, 134029.
- Li, W., Zheng, T., Ma, Y., Liu, J., 2019b. Current status and future prospects of sewer biofilms: Their structure, influencing factors, and substance transformations. *Sci. Total Environ.* 695, 133815.
- Li, X., Li, Y., Lv, D., Li, Y., Wu, J., 2020. Nitrogen and phosphorus removal performance and bacterial communities in a multi-stage surface flow constructed wetland treating rural domestic sewage. *Sci. Total Environ.* 709, 136235.
- Li, Z.Q., Kong, L.Y., Yang, L.F., Zhang, M., Cao, T., Xu, J., Wang, Z.X., Lei, Y., 2012. Effect of substrate grain size on the growth and morphology of the submersed macrophyte *Vallisneria spiralis* L. *Limnologia* 42, 81-85.
- Lu, J., Peng, H., 2012. Research progress in resistance of plants in *Iris*. *Acta Agr. Jiangxi* 24, 48-51.
- MacTavish, R.M., Cohen, R.A., 2017. Water column ammonium concentration and salinity influence nitrogen uptake and growth of *Spartina alterniflora*. *J. Exp. Mar. Biol. Ecol.* 488, 52-59.
- Marschner, P., Yang, C.H., Lieberei, R., Crowley, D.E., 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil Biol. Biochem.* 33, 1437-1445.
- Mateus, D.M.R., Pinho, H.J.O., 2020. Evaluation of solid waste stratified mixtures as constructed wetland fillers under different operation modes. *J. Clean. Prod.* 253, 119986.

- Mei, X.Q., Yang, Y., Tam, N.F.Y., Wang, Y.W., Li, L., 2014. Roles of root porosity, radial oxygen loss, Fe plaque formation on nutrient removal and tolerance of wetland plants to domestic wastewater. *Water Res.* 50, 147-159.
- Qin, K., Dong, X., Jifon, J., Leskovar, D.I., 2019. Rhizosphere microbial biomass is affected by soil type, organic and water inputs in a bell pepper system. *Appl. Soil Ecol.* 138, 80-87.
- Radhakrishnan, R., Baek, K.H., 2017. Physiological and biochemical perspectives of non-salt tolerant plants during bacterial interaction against soil salinity. *Plant Physiol. Bioch.* 116, 116-126.
- Ramond, J.-B., Welz, P.J., Cowan, D.A., Burton, S.G., 2012. Microbial community structure stability, a key parameter in monitoring the development of constructed wetland mesocosms during start-up. *Res. Microbiol.* 163, 28-35.
- Romero-Oliva, C.S., Contardo-Jara, V., Pflugmacher, S., 2015. Antioxidative response of the three macrophytes *Ceratophyllum demersum*, *Egeria densa*, and *Hydrilla verticillata* to a time dependent exposure of cell-free crude extracts containing three microcystins from cyanobacterial blooms of Lake Amatitlán, Guatemala. *Aquat. Toxicol.* 163, 130-139.
- Sheng, Q., Wang, L., Wu, J., 2015. Vegetation alters the effects of salinity on greenhouse gas emissions and carbon sequestration in a newly created wetland. *Ecol. Eng.* 84, 542-550.
- Singh, V.K., Singh, A.K., Singh, P.P., Kumar, A., 2018. Interaction of plant growth promoting bacteria with tomato under abiotic stress: A review. *Agr. Ecosyst. Environ.* 267, 129-140.
- Sun, H., Xu, S., Wu, S., Wang, R., Zhuang, G., Bai, Z., Deng, Y., Zhuang, X., 2019. Enhancement of facultative anaerobic denitrifying communities by oxygen release from roots of the macrophyte in constructed wetlands. *J. Environ. Manage.* 246, 157-163.
- Tadros, C.V., Treble, P.C., Baker, A., Hankin, S., Roach, R., 2019. Cave drip water solutes in south-eastern Australia: Constraining sources, sinks and processes. *Sci. Total Environ.* 651, 2175-2186.
- Vergani, L., Mapelli, F., Zanardini, E., Terzaghi, E., Di Guardo, A., Morosini, C., Raspa, G., Borin, S., 2017. Phyto-rhizoremediation of polychlorinated biphenyl contaminated soils: An outlook on plant-microbe beneficial interactions. *Sci. Total Environ.* 575, 1395-1406.
- Vymazal, J., 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. *Hydrobiologia* 674, 133-156.
- Wang, C., Liu, Z., Zhang, Y., Liu, B., Zhou, Q., Zeng, L., He, F., Wu, Z., 2018. Synergistic removal effect of P in sediment of all fractions by combining the modified bentonite granules and submerged macrophyte. *Sci. Total Environ.* 626, 458-467.

- Wang, J., Wang, S., 2019. Preparation, modification and environmental application of biochar: A review. *J. Clean. Prod.* 227, 1002-1022.
- Wang, Q., Ma, L., Zhou, Q., Chen, B., Zhang, X., Wu, Y., Pan, F., Huang, L., Yang, X., Feng, Y., 2019. Inoculation of plant growth promoting bacteria from hyperaccumulator facilitated non-host root development and provided promising agents for elevated phytoremediation efficiency. *Chemosphere* 234, 769-776.
- Wang, X., Huang, J. 2014. Principles and techniques of plant physiological and biochemical experiments. Beijing Higher Education Press.
- Wang, Y., Cai, Z., Sheng, S., Pan, F., Chen, F., Fu, J., 2020. Comprehensive evaluation of substrate materials for contaminants removal in constructed wetlands. *Sci. Total Environ.* 701, 134736.
- Xu, Y., Wang, G., Jin, J., Liu, J., Zhang, Q., Liu, X., 2009. Bacterial communities in soybean rhizosphere in response to soil type, soybean genotype, and their growth stage. *Soil Biol. Biochem.* 41, 919-925.
- Yan, J., Jiang, T., Yao, Y., Wang, J., Cai, Y., Green, N.W., Wei, S., 2017. Underestimation of phosphorus fraction change in the supernatant after phosphorus adsorption onto iron oxides and iron oxide–natural organic matter complexes. *J. Environ. Sci.* 55, 197-205.
- Yang, Y., Zhao, Y., Liu, R., Morgan, D., 2018. Global development of various emerged substrates utilized in constructed wetlands. *Bioresour. Technol.* 261, 441-452.
- Zhai, W., Ding, J., An, X., Wang, Z., 2020. An optimization model of sand and gravel mining quantity considering healthy ecosystem in Yangtze River, China. *J. Clean. Prod.* 242, 118385.
- Zhao, D., Chen, C., Lu, Q., Zhang, M., Zhang, H., An, S., 2020. Combined use of cold-season and warm-season macrophytes in floating constructed wetlands to increase nitrogen removal in the early cold season. *J. Clean. Prod.* 254, 120054.
- Zhao, D., Lv, L., Liu, Z., Zhang, M., An, S., 2018a. The carbon source function of constructed wetland macrophytes and its improvement strategy. *Acta Ecol. Sin.* 38, 5961-5969.
- Zhao, D., Zhang, M., Liu, Z., Sheng, J., An, S., 2018b. Can cold-season macrophytes at the senescence stage improve nitrogen removal in integrated constructed wetland systems treating low carbon/nitrogen effluent? *Bioresour. Technol.* 265, 380-386.
- Zheng, Y., Wang, X., Dzakpasu, M., Zhao, Y., Ngo, H.H., Guo, W., Ge, Y., Xiong, J., 2016. Effects of interspecific competition on the growth of macrophytes and nutrient removal in constructed wetlands: A comparative assessment of free water surface and horizontal subsurface flow systems. *Bioresour. Technol.* 207, 134-141.

Zheng, Y., Wang, X., Xiong, J., Liu, Y., Zhao, Y., 2014. Hybrid constructed wetlands for highly polluted river water treatment and comparison of surface- and subsurface-flow cells. *J. Environ. Sci.* 26, 749-756.

Zhu, Y., Jiang, C., Zhu, L., Du, G., Gao, X., Chen, H., Li, F., Zhang, H., Zhang, X., Qin, W., Li, Y., 2019. Accumulation characteristics and release regularity of nutrients in sediments of a surface-flow constructed wetland after long-term operation. *Environ. Sci.* 40, 2764-2772.

Zou, X., Zhang, H., Zuo, J., Wang, P., Zhao, D., An, S., 2016. Decreasing but still significant facilitation effect of cold-season macrophytes on wetlands purification function during cold winter. *Sci. Rep.* 6, 27011.

Figures

Figure 1

The removal efficiencies of salt, TN, TP and COD for SFCWs with soil, sand and gravel as the substrate at 0.8 ‰ to 6.0‰ influent water salinity.

Figure 2

The ecophysiological parameters of the macrophytes at the four influent water salinities (in Fig. 2A, the dry biomass above and below the indicator line is the aboveground and underground partment, respectively. In Figs. 2D and 2E, the influent water salinity of 0.1‰ is the water characteristic at the preculture period, i.e., the pond water for the synthetic wastewater).

Figure 3

The rhizosphere bacterial diversity as represented by the Shannon index and observed species number at the four influent salinities.

Figure 4

The percent relative abundances at the phylum (A) and genus (B) levels (SO, SA and GR are the treatments with soil, sand and gravel as the substrates, respectively, and P1, P2, P3 and P4 are influent water salinities of 0.8‰, 1.5‰, 3.0‰ and 6.0‰, respectively).

Figure 5

RDA of the SFCWs using the pollutant REs and macrophyte physiological and ecological indexes and rhizosphere bacterial parameters at influent salinities of 0.8‰ (A), 1.5‰ (B), 3.0‰ (C) and 6.0‰ (D) (AB, UB, RA, CAT, SOD, SPAD, Sobs and Shannon are aboveground biomass, underground biomass, root activity, root CAT activity and root SOD activity, leaf chlorophyll concentration in SPAD value, Shannon index of rhizosphere bacteria, and observed species number of rhizosphere bacteria).

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