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1	New insights for enhancing the performance of constructed wetlands
2	at low temperatures
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Abstract: Constructed wetlands (CWs) have been widely utilized for various types of wastewater treatment due to their merits, including high cost-effectiveness and easy operation. However, a few intrinsic drawbacks have always restricted their application and long-term stability, especially their weak performance at temperatures under 10°C (low temperatures) due to the deterioration of microbial assimilation and plant uptake processes. The existing modifications to improve CWs performance from the direct optimization of internal components to the indirect adjunction of external resources promoted the wastewater treatment efficiency to a certain degree, but the sustainability and sufficiency of pollutants removal remains a challenge. With the goal of optimizing CW components, the integrity of the CW ecosystem and the removal of emerging pollutants, future directions for research should include radiation plant breeding, improvements to CW ecosystems, and the combination or integration of certain treatment processes with CWs to enhance wastewater treatment effects at low temperatures.

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- Keywords: Constructed wetland; low temperature; performance enhancement;
- 42 ecosystem; radiation breeding.

# 1. Introduction

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44	In the narrow sense, wetlands are ecotones with high biodiversity that exist
45	between terrestrial ecosystems and aquatic ecosystems, and their surfaces are wet or
46	often accumulate water (Junk et al., 2013). The global area of wetlands estimated from
47	the Ramsar Convention and United Nations Environment Program is between 1.52*109
48	hm² and 1.62*109 hm² (Davidson et al., 2018; Gong et al., 2010; Zheng et al., 2015). As
49	one of the three major ecosystem types worldwide and known as being "the earth's
50	kidney" (Janse et al., 2019), the wastewater could be purified in wetlands through a
51	series of processes (sediment entrapment, nutrient absorption, and toxic substance
52	degradation) based on the synergistic action of microbe, soil, and plant. Moreover, on
53	the one hand, the wetland is the source of water storage, and provides natural resources
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54	such as peat, reed, and medicinal materials in human production and life. On the other
55	hand, it has the excellent characteristic of genetic diversity and provides a suitable
56	habitat and breeding area for aquatic animals and plants (An and Finlayson, 2018). The
57	function of the wetland ecosystem at the evaluation level of the ecological environment
58	and social economy creates an annual value up to 14,000 dollars/hectare, which is 7
59	times and 160 times the value of the tropical rain forest and the farmland ecosystem,
60	respectively (Davidson et al., 2019; Junk et al., 2013; Kumar and Dutta, 2019; Moomaw
61	et al., 2018). However, the Wetland Extent Trends index have suggested that the natural
62	wetlands declined globally on average by about 30% between 1970 and 2008 (Davidson
63	and Finlayson, 2018; Dixon et al., 2016). Based on imitation of the structure and

64	function of natural wetlands and mitigation of wetland destruction (Davidson, 2014; Xu
65	et al., 2019), constructed wetlands (CWs) concentrated on wastewater treatment have
66	received increasing attention in recent decades (Arden and Ma, 2018; Feng et al., 2019;
67	Wang et al., 2018).
68	CWs are an integrated ecosystem for efficiently removing pollutants by synergetic
69	processes, including plant uptake, microbe assimilation and substrate adsorption (Wu et
70	al., 2015). The advantages of high cost-effectiveness and easy operation make CWs
71	appropriate for wastewater treatment in regions without public wastewater systems or
72	regions that are economically underdeveloped (Wu et al., 2015; Wu et al., 2018). In
73	China, the construction area of CWs (674.59 million hectares in 2014, accounting for
74	12.63% of the total wetland area in China) based on the local geographical conditions,
75	climatic factors, and ecological landscapes have developed rapidly in recent years (Fig.
76	1) (Zhou et al., 2014). Particularly due to their low construction costs (one-third to
77	one-half the cost of a wastewater treatment plant) and operation maintenance costs
78	(0.05-0.2 yuan/ton wastewater, compared to 0.7-1.5 yuan/ton wastewater in a
79	wastewater treatment plant) (Yu et al., 2018), CWs play an irreplaceable role in the
80	wastewater treatment and economic development of the developing countries like China
81	and India (Gorgoglione and Torretta, 2018; Semeraro et al., 2015).
82	According to an analysis of the distribution of these wetlands, however, they are
83	mainly located in the mid-low latitudes, and only a small number of wetlands are located
84	in the high latitudes (An and Finlayson, 2018). Because of the ecological attributes of

85 CWs, climate conditions have always been a significant factor in the unreliable water 86 treatment of CWs (Hwang and Oleszkiewicz, 2007; Stottmeister et al., 2003; Xie et al., 2003; Zietzschmann et al., 2015). Previous studies have shown that the removal 87 88 efficiencies of ammonia nitrogen (NH<sub>4</sub><sup>+</sup>-N), total nitrogen (TN) and total phosphorus (TP) at low temperatures were reduced by 15%, 45% and 16%, respectively, compared 89 to those observed at the optimal temperature (25°C) (Song et al., 2006; Taylor et al., 90 91 2011; Zhao et al., 2019). When microbial activity and plant growth are affected by low 92 temperatures, the processes of pollutant transformation and mass transfer in CWs are 93 retarded, so the declining efficiency of the ecosystem function and the insufficient performance period limit the removal efficiency of CWs as well as their promotion and 94 95 application (Dixon et al., 2016). Therefore, it is necessary to promote the construction 96 value of CWs by achieving year-round stable operation performance, and enhancing the 97 performance of CWs at low temperatures is undoubtedly a significant issue demanding 98 a prompt solution. 99 This paper elucidated the mechanisms affecting the operation performance of CWs 100 and summarized the existing strategies for the optimization of internal components and 101 the improvement of external operation conditions of CWs in order to enhance

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Fig. 1 The distribution of CWs in administrative regions of China (The site and size of

wastewater treatment at low temperatures. Meanwhile, the new pathways necessary and

helpful for future applications of CWs at low temperatures were discussed.

106	circles indicates provincial capital and area respectively).
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108	2. Impact of low temperatures on constructed wetlands performance
109	2.1. Pollutant removal pathways of constructed wetlands
110	The removal pathways (Fig. 2) of common pollutants (nitrogen and phosphorus) in
111	CWs were mainly attributed to the processes of plant uptake, substrate adsorption, and
112	microbe assimilation, which occupied different proportions in pollutants removal due to
113	their respective removal mechanisms (Coban et al., 2015). However, at low
114	temperatures, these processes were affected directly or indirectly by a variety of
115	variables including the oxygen transfer efficiency of the plant, the adsorption and
116	sedimentation velocity of the substrate, the microbial metabolism rate, the operational
117	mode, and the dissolved oxygen concentration. Although low temperatures may result
118	in a seemingly favorable change in CWs like the increase of dissolved oxygen, various
119	routes of pollutant removal in CWs were adversely affected by low temperatures
120	(Blanco et al., 2016).
121	
122	Fig. 2 Main pathways of pollutants removal in CWs.
123	(1) OP: Organophosphorus; DP: Dissolved phosphorus; IP: Inorganic phosphorus; PP:
124	Particulate phosphorus. (2) Account for the proportion of pollutants removal: ①
125	5.4-20.1% N, 4.8-22.3% P; ② 4.5-8.3% N, 36.2-49.7% P; ③ 89-96% N.

# 2.2. Impact of low temperatures on microbes

As the major biological community responsible for pollutant degradation and assimilation (Petersen et al., 2015), microbe was perceived as making great contributions to CWs; the transformation processes of organic matter and nitrogen compounds are primarily completed in the aerobic and anaerobic zones of CW wastewater via microbial metabolic activities (Bai et al., 2014; Faulwetter et al., 2009). However, (1) at low temperatures, the microbial activity and metabolic rate in the CW systems were reduced, which seriously impeded the heterotrophic bacteria in decomposing organic pollutants (Lin et al., 2015); (2) the reaction efficiency of nitrification (optimal temperature of 20-30°C) dropped rapidly while the temperature was 15°C, and the reaction almost stopped at 5°C (Jurado et al., 2009); and (3) simultaneously, as the most effective way to denitrify (85% of nitrogen) in CWs, the reaction rate of denitrification (optimal temperature of 5-40°C) also decreased at 15°C, thus resulting in a decrease in the operational efficiency of CWs (Du et al., 2018).

# 2.3. Impact of low temperatures on plants

Plant was an indispensable component in CW treatment, as they could utilize the nitrogen and phosphorus nutrients from wastewater for their own growth and conduct the plant-mediated oxygen transfer for organic carbon oxidation and nitrification processes (Březinová and Vymazal, 2015). In general, the temperature of plant growth

was in the range of 4-36°C, while the fiducial temperature was 5-10°C (Huang et al., 2013). However, (1) the metabolic processes of plants, such as photosynthesis, respiration and transpiration, were weakened at low temperatures, which affected the synthesis and transport of organic matter (Van de Moortel et al., 2010); (2) the oxygen supply to plants was restricted at low temperatures, and the voids connected with substrate and air affected the radial oxygen loss because of the decreasing root activity, thus resulting in a reduction in reoxygenation, which then affected the assimilation of nitrogen pollutants (Ren et al., 2016); and (3) since soil and air temperature were also directly influenced at low temperatures, the amounts of available nutrients, such as ammonium, nitrate and phosphate, that could be directly adsorbed or assimilated by plants from the CW soil were reduced dramatically. Moreover, the ice crystals formed in dormant plants caused withering and even death at extreme low temperatures, after the processes of plasmalemma breakdown and protein inactivation and denaturation, therefore reducing the removal performance of the plant (Stottmeister et al., 2003).

# 2.4. Impact of low temperatures on the substrates

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The substrate, a crucial parameter in CW treatment, furnished most of the physical, chemical and biological reaction interfaces in CWs (Zhou et al., 2018). Commonly used types of substrate in CWs were including natural materials, industrial waste, and synthetic materials (Yang et al., 2018). Not only did substrate provide a stable attachment surface for the microbe growth and a growing medium for aquatic plants but

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they also furnish the high hydraulic conductivity avoiding short-circuiting of wastewater (Stefanakis and Tsihrintzis, 2012). The nonbiological interactions between the phosphorus and the substrate, such as the process of chemical deposition between phosphate and cation (e.g., Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Al<sup>3+</sup>), was deemed to be the main mechanism of phosphorus removal (36.16-49.66%) in CWs (Kivaisi, 2001).

Akratos and Tsihrintzis (2007) indicated that the mean PO<sub>4</sub><sup>3-</sup> and TP removal efficiencies under 15°C were decreased by 28.5% and 28.3%, respectively, compared with those above 15°C. Citing zeolite as an example, the temperature affected both phases (internal and external diffusion phases of particles) of substrate adsorption (Yang et al., 2018). During the adsorption process at low temperatures, the ability of phosphorus in wastewater to overcome the liquid film resistance of the zeolite surface was weakened, which effectively migrated the phosphorus along the pores of zeolite into the inside, thereby reducing the surface area of the adsorption site (McCarey et al., 2004; Postila et al., 2015). Moreover, external low temperatures reduced the adsorption capacity of the wetland bed because the process of phosphorus adsorption was an endothermic reaction. The viscosity of wastewater increased and the rate of water molecules entering the plant root zone decreased, resulting in a decline in oxygen transfer efficiency and in physiological activity of plant and microbe (Pang et al., 2015). However, Sani et al. (2013) showed that phosphorus removal did not depend on the temperature, based on seasonal variations under changes to hydraulic retention time (HRT) and loading rate, because physical-chemical reactions, rather than biological

action, were principally responsible for TP removal. It should be clarified that the particular phosphorus did not dissolve well in the substrates that were not saturated by phosphorus or other compounds competing of adsorption sites. Likewise, Jenssen et al. (1993) ascribed the 98% phosphorus removal to the adsorption force of the porous medium and the iron oxides in soil.

# 3. Modifications to enhance constructed wetlands performance at low temperatures

Aiming to improve the low removal rate of CWs at low temperatures, researchers have carried out many modifications based on the internal space components (microbial activity, plant uptake, substrate addition) or external operational factors (heat loss, operating situation, and dissolved oxygen) of CWs in recent years in response to the effects of cold stress on CW performance (Fig. 3). Direct modifications to the CW components included the utilization of microbial inoculum and bioturbation for the promotion of microbial activity (bio-augmentation), seasonal planting collocation systems and cold-resistant plants to promote plant uptake (plant configuration), and supplements of plant straw and plant-based biochar to create a favorable anaerobic environment and encourage ion exchange (substrate property). Indirect modifications based on the CW operation included adding mulch material and building greenhouses (isolation and heat preservation), imposing batch-type flow for the alternation of aerobic-anaerobic treatment conditions and adjusting HRT/ hydraulic loading rate

207	(HLR)	parameters	(treatment	condition),	and	for	improving	dissolved	oxygei
208	concent	tration (artific	cial aeration)	).					

# Fig. 3 Modifications were responding to the cold stress on CW performance at low

211 temperatures.

213 3.1. Direct modifications

# 214 3.1.1. Bio-augmentation

Microbial degradation was the main route to removing pollutants in CWs (Wood et al., 1999). However, the growth of microbes in CWs was very restricted at low temperatures (Jurado et al., 2009; Liu et al., 2017). Xu et al. (2018) found that *Psychrotrophs* were adapted to domestic wastewater due to their better adaptation for temperature fluctuations. Aiming to attain the approximate degree of microbial metabolism compared with that under normal temperatures in CWs at low temperatures, Xu et al. (2017) revealed that the *Pseudomonas putida* Y-9 screened from long-term flooded paddy soil (**Table 1**) had an excellent denitrification capacity (1.60 mg nitrate nitrogen (NO<sub>3</sub>-N)/L/h, 1.83 mg nitrite nitrogen (NO<sub>2</sub>-N)/L/h) for treating wastewater at 15°C, especially the wastewater containing high concentrations of nitrogen. Immobilized microorganism technology was also a way to degrade organic matter by increasing redox potential through cold-adapted bacteria placed in stable carrier material

227	(Ben et al., 2009; Isaka et al., 2012; Zhang et al., 2011), but the removal rate by directly
228	adding microbes to CWs would be limited by the decomposition of the gelatin and the
229	adaptability of the bacterial strain itself. Bio-augmentation was an effective alternative,
230	as it provided the microbe with particular functions at a sufficient amount (Yeh et al.,
231	2010). Compound microbial agents formed a new microflora balance in situ by
232	transforming the intrinsic species homogeneity of nitrogen-related bacteria in the soil
233	microbial community structure of CWs (Zhao et al., 2016). The addition of multichannel
234	biological fillers including salt-tolerant bacteria, photosynthetic bacteria,
234	biological liners including sait-tolerant bacteria, photosynthetic bacteria,
235	low-temperature dephosphorization and nitrogen-removal bacteria showed the 35.4%
236	$\mathrm{NH_4}^+\mathrm{-N},\ 33.6\%$ TN, 28% TP, and 30% chemical oxygen demand (COD) removal
237	efficiencies in surface-flow CWs under the double stresses of high salt and low
238	temperature. Although the microbial richness of the system was reduced, the major
239	microbial species present were not significantly different (Duan et al., 2016).
240	The nitrogen and phosphorus removal of CWs with earthworms were 2-5% and
241	12% higher than those without earthworms, respectively (Li et al., 2011). It was
242	reported that the microbial-earthworm ecofilters (MEEs) system had the advantages of a
243	microporous structure, and higher porosity and specific surface area, and could be
244	applied to CWs for COD removal and denitrification at low temperatures (Huang et al.,
245	2014; Wang et al., 2011; Zhao et al., 2012; Zhao et al., 2014). In terms of the economic
246	effectiveness, MEEs had a lower operational and maintenance cost in treating
247	wastewater as no requirements on experienced labor and a little energy supplement on

248	pumps. Meanwhile, as a feasible option with economy for earthworm breeding, MEEs
249	could create the value up to almost \$ 40 (1,000 earthworms) in market of Australia
250	(Sinha et al., 2008). Even more important, the total suspended solids and COD could be
251	removed more efficient in MEEs than another biofilter without worms in domestic
252	wastewater treatment. It can be applied into as pre-treatment for high concentration of
253	COD and suspended solids of wastewater, which was in favor of the mitigation for
254	substrate clogging and organic load in CWs (Xing et al., 2010).
255	Moreover, earthworms could help not only in enhancing the mass transfer process in
256	the vertical direction through bioturbation but also in improving the food chain link with
257	aquatic animals (Chiarawatchai and Nuengjamnong, 2009; Wu et al., 2013).
258	Furthermore, the earthworm Eisenia fetida could promote the wetland plants growth
259	due to the variation of nitrification potentials in rhizosphere (Xu et al., 2013). From this
260	point of view, the application of earthworm in CWs is to the benefit of nutrients uptake
261	and recycling values of plants. Similarly, Tubifex tubifex could remain alive at low
262	temperatures and maintain high survival; their feces were also excellent manure and an
263	adequate carbon source for denitrification. Its stimulations of bioturbation activity for
264	microbial communities indirectly impacting the nitrogen cycle via mediated redox
265	reactions and the usability of O <sub>2</sub> . Kang et al. (2016) increased the TN and NO <sub>3</sub> -N
266	removal efficiencies of wastewater by 22.92% and 40.87%, respectively, through the
267	utilization of benthos in CWs. The use of animals was a good idea for better CW
268	treatment performance at low temperatures through the synergistic effects among plant,

269 microbe, and animal.

Table 1 Modifications were for enhancing CWs performance at low temperatures.

# 3.1.2. Plant configuration

The characteristics of plant configuration were mainly based on suitability for the local climatic environment, the size of the root system, the nutritional level of the water body, strong purification ability, anti-freeze characteristics, anti-fouling performance, and anti-impact load abilities (Allen et al., 2002). At present, according to the remarkable removal efficiencies of various plant species, the common plants applied in CWs included *Phragmites australis*, *Typha orientalis*, *Water hyacinth*, *Acorus calamus*, etc. (Brisson and Chazarenc, 2009; Chen et al., 2014). To deal with the influence of low temperature acting on CWs, a few plants have been cultivated for higher nutrient uptake. For instance, the evergreen *Lolium perenne* achieved 92.5% NH<sub>4</sub><sup>+</sup>-N removal (0.2-2.6°C) based on its strong nitrogen absorption abilities (Ren et al., 2016). Due to its efficient intake of nitrogen and strong viability, *Carex aquatilis* screened in tundra wastewater treatment wetlands showed a 78% decrease in NH<sub>3</sub>-N concentration at 0-5°C through its photosynthetically active radiation pattern (Yates et al., 2016).

Interestingly, the seasonal plant configuration was originally carried out on the basis of single plants applied to CWs. *Potamogeton crispus* enhanced the formation of

cold-adapted ammonia-oxidizing bacteria by increasing the oxygen concentration in the wastewater and increasing oxygen transfer to plant roots (Fan et al., 2016); a seasonal plant collocation system (SPCS) consisting of *Potamogeton crispus* and *Phragmites australis* achieved plant growth and nutrient uptake sustainably during all seasons in northern China and achieved better removal NH<sub>4</sub><sup>+</sup>-N and TP efficiencies (18.1% and 17.6%, respectively) than the group that only cultivated *P. australis* in winter. SPCSs were of prime strategic importance for the sustainable operation of CWs located in areas at low temperatures (Zhang et al., 2017).

# 3.1.3. Substrate supplements

The choice of the substrate in CWs was determined by factors such as mechanical strength, stability, specific surface area, porosity and surface roughness (Yang et al., 2018). To mitigate the adverse influences of low temperatures on wastewater treatment in CWs, scholars have used additional materials as supplementary enhancements of nitrogen removal. Cao and Zhang (2014) found that rice straw combined with an ecological floating bed could provide a favorable anaerobic environment and extra carbon sources for denitrification reactions and achieved removal efficiencies of 78.2% TN and 62.1% NO<sub>3</sub>-N in CWs (9.5-14.5°C) because a thick biofilm was formed by the decomposition of the supplemental material in eutrophic water bodies. Moreover, the natural rice straw was less expensive than nonbiodegradable materials. On the strength of the microscopic properties of the porous stem leaves of CW plants, Li et al. (2018)

prepared Arundo donax-derived biochar and filled it in substrate for reinforcing nitrogen
adsorption. The 2 mg·g-1d-1 release rate of the dissolved organic matter produced in the
biochar helped increase the TN removal (85.62%) efficiency to approximately 50%
higher than that in the no-biochar treatment in subsurface flow CWs (5.38±4.95°C).
This botanical biochar with high porosity and a large surface area due to the porous
structure of the hydrophyte provided numerous attachment sites and bioavailable carbon
for denitrifying bacteria, which was beneficial to nitrogen transformation because of the
anaerobic environment formed on the surface of the biochar. Moreover, compared with
internal carbon sources, the appropriate dose of additional carbon sources was
undetermined, and excessive doses would cause higher costs and adverse denitrification
performance. Based on the concepts of plant management and resources recovery, the
use of biochar was effective and necessary for enhancing CW performance at low
temperatures; it also allowed the recycling of waste plant biomass resources and the
prevention of secondary pollution of water bodies (Li et al., 2018).
In addition, Zhao et al. (2019) added iron-based material to a vertical
subsurface-flow CW in order to enhance ion exchange and microbial reactions; this
treatment achieved 65.62% TN removal efficiency in low carbon-nitrogen ratio
wastewater (0-10°C). In terms of phosphorus removal, in addition to using conventional
sand filter media, pine branches, activated sludge of active substances, iron slag and
limestone were added to enhance the exchange between phosphorus and cations of Ca <sup>2+</sup>
and Fe <sup>3+</sup> . However, this complementary effect was still needs the testing to determine

- whether it is suitable at low temperatures (Yang et al., 2018). Moreover, strengthening the degradation of microbes attached to substrates should also be considered for solving the problem of substrate desorption of phosphorus (Mietto et al., 2015).
- 333 *3.2. Indirect modifications*

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3.2.1. Insulation and heat preservation

Various mulch materials, such as snow, straw, breathable film (polyvinyl chloride), carbonized reeds, and organic fillers, have been applied in horizontal subsurface flow CWs at low temperatures to maintain the temperature in a stable range or to prevent freezing to some extent through natural or artificial insulation (Akratos and Tsihrintzis, 2007). Materials covering the surface of CWs could reduce the energy lost due to wastewater evaporation, transportation and flow, and protect the CW internal microbe from the adverse effects of external low temperatures (Pang et al., 2015). Carbonized reed straw added to CWs maintained the CW wastewater temperature at 11-13°C (Yin and Shen, 1995). Similarly, Zhang et al. (2006) showed that adding a layer of plastic film on the surface of a CW maintained the wastewater temperature at range of 15-18°C, which greatly reducing the depth of frozen soil and increasing the mean removal rates of NH<sub>4</sub><sup>+</sup>-N and COD by 38.2% and 17.6%, respectively. However, the introduction of the plastic film influenced the oxygen transfer rates, and the laying work was complicated although its cost was inexpensive. Moreover, the mulch film was breakable and difficult to maintain and would cause white pollution if not removed in

time. The characteristics of coverings used for CW insulation should decompose
substantially and without any secondary organic loading and have a neutral pH, high
fiber content, balanced nutrient composition, good heat insulation and high moisture
retention capacity (Wu et al., 2015). In addition, Wallace et al. (2003) found a naturally
insulating air layer for heat preservation between the water surface and the ice surface at
-4-18°C in Canada. Although ice cover did not cause secondary pollution problems in
the same way as plant cover, it needed to be checked periodically. However, snow and
ice cover are not reliable enough to insulate CWs during cold periods with finite snow
accumulation. Moreover, when the ice surface melted in large areas because of
temperature fluctuations, it was a complicated operation due to the frozen layer needs to
be reformed. In addition to providing insulation and heat preservation, optimally
designed greenhouse (to maintain the wastewater temperature above 8°C) for raising the
temperature of bioecological horizontal subsurface flow CW systems achieved the
annual mean removal rates of COD (85.01%), NH $_3$ -N (70.98%) and TP (36.48%) (Gao
and Hu, 2012). On the one hand, especially in winter, the greenhouse could be used for
communication and entertainment towards the local people. On the other hand, it was
also an ideal place for rice-cultivating in spring as well as brought in income for
indigenous people. In terms of economic benefits, the greenhouse-structured wetlands
have the great latent value for rural area in possession of the aquaculture industry (such
as soft-shelled turtle greenhouse cultivation) (Hu et al., 2012; Wu et al., 2014).

# 3.2.2. Changes in operating conditions

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The oxygen transfer rate was much higher in batch-type flow CWs (water supply and drainage at regular intervals for better oxygen capacity and continuously forming aerobic and anaerobic environments) than in conventional CWs at 12-25°C (Mesquita et al., 2017). Zhou et al. (2007) revealed that the NH<sub>3</sub>-N and TN removal rates (5-10°C) by batch-type flow CWs reached 85.86% and 48.44%, respectively. Compared with the continuous flow CW, better COD removal efficiency, microbe respiration intensity, and hydrogenase activity were obtained. However, Zhang et al. (2012) believed that the higher DO level of the CW system inhibited the denitrification performance in the interior of the system although the batch-type operation mode did improve the denitrification ability and pollutant removal stability of the CWs at low temperatures. Moreover, batch-type flow CWs always restricted TN removal regardless of the HRT values. Generally, a long HRT allows sufficient interaction between pollutants and wastewater. The HLR, which is important for microbial activity, organic matter degradation, and nutrient conversion, also affects the transport of pollutants and oxygen environment inside the CW (Põldvere et al., 2009; Rehman et al., 2017). Zhang et al. (2006) increased the removal rates (5-15°C) of NH<sub>4</sub>+-N and COD from 14% to 39% and 20% to 31%, respectively, after the hydraulic load was reduced from 30 cm/d to 15 cm/d. Conversely, the design of tower-hybrid CWs showed some nitrogen removal capacity at HLRs of 16 cm/d and 32 cm/d at mean temperatures close to 8°C (5.5°C at the lowest)

(Ye and Li, 2009). These different results may be because hydraulic efficiency varies between different CW configurations. In addition, Solano et al. (2004) reported that the highest organic removal rate was at a low HLR and a high HRT within a certain range (150, 75 mm/d and 1.5, 3.0 d, respectively), and the higher HRT (>3.5 d) contributed to an increase in organic matter removal (>70%) with a minimum mean temperature of 5°C and a maximum mean temperature of 15°C (Garfi et al., 2012).

# 3.2.3. Artificial aeration

Nitrification completion was closely related to the concentration of oxygen in CWs (Wu et al., 2014). However, in addition to direct reoxygenation from the atmosphere, the remaining amount of oxygen inside the CW was small since most of the oxygen derived from photosynthesis was used for the plant root respiration (Wang and Li, 2017). Artificial aeration could achieve 40-60% for TN removal efficiency by stimulating heterotrophic bacterial activity (Fan et al., 2013). Tao et al. (2010) found that eight hours of artificial aeration per day was enough to eliminate a large amount of NO<sub>3</sub>-N accumulation in continuous aerated subsurface flow CW effluent at 6.4±2.2°C. Continuous aeration significantly enhanced the conversion ratio of NH<sub>4</sub>+-N to NO<sub>3</sub>-N, but a continuous oxygen supply inhibited the subsequent denitrification due to the rapid expense of the carbon sources in the influent and the lack of an efficient hypoxia zone, while the denitrification process was responsible for more than half of the nitrogen removal (Maltais-Landry et al., 2007). The high operation costs of electromechanical

devices for continuous aeration was also a potential problem, but the cost may be justified in mountainous regions where the space availability was limited (Foladori et al., 2013). However, the CW operation mode with intermittent aeration following step feeding effectively perfected the process of balancing denitrification with an effective nitrifying process; the additive carbon source supply in the new inflow and the alternation between aerobic and anaerobic environments achieved NH<sub>4</sub><sup>+</sup>-N and TN removal efficiencies of 96% and 82%, respectively, at 14.7°C (Fan et al., 2013). The effect of step feeding on NO<sub>3</sub><sup>-</sup>-N accumulation was perhaps more prominent in continuously aerated CWs, but it should be weighed against the higher energy costs.

# 4. Future directions for the application of constructed wetlands at low

# temperatures

The aforementioned modifications for enhancing CW performance at low temperatures did make progress through the optimization of operations and spatial configuration in the CW interior and of the energy input and structural insulation in the CW exterior. With the goals of optimizing the CW components, perfecting the integrity of CW ecosystems and addressing emerging persistent organic pollutants (POPs), several future directions for CW application at low temperatures, such as the breeding of cold-resistant plants, bio-manipulation, and CWs integrated or combined with other treatments, were proposed for sustainable and diverse pollutants removal.

430 4.1. Breeding cold-resistant plants

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Using CW plants that were adapted to the low-temperature environments would help sustain the absorption of wastewater nutrients in all seasons (Fan et al., 2016). In terms of the potential economic and technological effects, strengthening the breeding and cultivation of very cold-resistant plants was a popular trend (Kumar Meena et al., 2015). The generation of cold-resistant plants occurred mainly through the three pathways discussed below by changing the expression of the enzyme system or the hormone regulatory system and through gene mutation, respectively (Van Doom et al., 2013). (1) Cold acclimation, i.e., appropriate low-temperature treatment of germinated seeds or seedlings by artificial or natural methods. Zhang et al. (2016) induced Mg-protoporphyrin IX (Mg-Proto IX) accumulation in Arabidopsis by use of the glutamate and MgCl<sub>2</sub> and found that Mg-Proto IX enhanced non-enzymatic antioxidants by activating antioxidant enzymes to maintain redox balance under cold stress. This processing mode could increase intracellular antioxidant enzyme activity and endogenous antioxidant levels (Calvo-Polanco et al., 2016; Dorffling et al., 1997), which alleviated the lipid peroxidation of membrane proteins and membranes caused by low temperature stress (Raju et al., 2018). (2) Chemical mutagenesis, i.e., exogenous ABA treatment increased the cold resistance of plants at normal temperatures while also inducing the expression of a variety of cold-stress genes to produce corresponding proteins (Huang et al., 2017). This was the process that increases the expression of

451	glycoproteins related to cell signal recognition at low temperatures and actuated the
452	accumulation of a few cold-resistant substances that protect the biofilm in cells, such as
453	proline, abscisic acid, and soluble sugars (Koehler et al., 2012). Moreover, Duan et al.
454	(2019) revealed that the biosynthesis of flavonoids and phenylpropanoids was enriched
455	in Lycopersicon esculentum Mill. under cold stress through analysis of the Kyoto
456	Encyclopedia of Genes and Genomes pathways. (3) Radiation breeding induced plant
457	mutations by X-rays, gamma-rays, ultraviolet rays, etc. to obtain new varieties.
458	Radiation frequently generates point mutations that could effectively improve a single
459	trait while maintaining the other excellent characteristics of a plant. The breeding
460	procedure was also a relative short cycle and overcomes incompatibilities from distant
461	hybridization (Ha et al., 2014).
462	These measures were used in the agricultural field to increase the yield of crops or
463	to mitigate the adverse effects of climatic factors. Whether these pathways could
464	generate functional genes or induce protein synthesis in cold-resistant plants effectively
465	for pollutants uptake from wastewater of CW at low temperatures, it needs to be
466	explored in future researches (Stamm et al., 2011; Zhang et al., 2014).

# 4.2. Perfecting the ecosystem of constructed wetland

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The ecosystems of natural wetlands were more complex and stable than CW ecosystems (Liu et al., 2017). The more improved a CW ecosystem was, the more stable the purification effect would be. Based on the reinforced synergy mechanism of the

seasonal application of CW organisms, an "(anti-pollution and cold-resistant)

plant-benthos-microbe" synergistic enhancement CW was established.

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First, the allocation of space for seasonal plantings (such as *Phragmites australis* and Potamogeton crispus) could help plants take in pollutants sustainably for growth in all seasons and increase the NH<sub>4</sub><sup>+</sup>-N (18.1%) and TP (17.6%) removal efficiencies compared with those in CWs planted in one species. Plant roots not only supply oxygen and attachment points for microbes but also provide oxygen, food, and habitat for benthos (Zhang et al., 2017). Second, the addition of benthos (such as freshwater mussels, Tubifex tubifex, Viviparidae and Chironomidae larvae) could provide carbon dioxide and carbon from their feces for plant photosynthesis and microbial denitrification, respectively. Meanwhile, with the function of cold-resistant benthos, the process of ingestion and transformation of pollutants would further promote the TN and NO<sub>3</sub>-N removal efficiencies by 22.92% and 40.87%, respectively (Kang et al., 2016). Last, as the main component for wastewater purification in CWs, microbe activity was activated due to the preferable DO environment created by seasonal plant collocation and bioturbation by cold-resistant benthos. Moreover, the anaerobic environment provided by the enteric canals of benthic organisms was also beneficial to denitrification. This "organism loop" CW structure (Fig. 4) could be applied to the typical wetland situation at low temperatures in winter in northern China (Fan et al., 2016; Kang et al., 2016; Zhang et al., 2017).

The next studies for exploring the "organism loop" system at low temperatures in

order to obtain more stable and sustainable pollutant removal efficiency should be related to the synergy between different cold-resistant plants in CWs. Moreover, the potential utility of benthonic animals such as Tubifex tubifex and freshwater mussels should be further tested for the stimulation dynamics of microbial processes, which benefited nitrogen transformation towards denitrification in CWs at low temperatures. In addition to the optimization of CWs for plants and animals, from the aspect of long-term stable pollutant removal efficiency, the function of the microbial population structure adapted to a low-temperature environment derived from a microbial inoculant needs to be screened further according to the metabolic characteristics and growing conditions of the microbes (Xu et al., 2017). For example, previous studies indicated that bio-augmentation, e.g., plant growth promoting rhizobacteria (PGPR), promoted the phosphorus uptake of plant since its secretion (such as extracellular phosphatase or organic acids) could convert the insoluble phosphate in soil into dissolving phosphorus that could be absorbed by plant. From this point of view, not only could it alleviate the clogging of phosphorus adsorption on surface of substrate, but also manifest the advantages of economic value in resources recovery of CW plant (Rehman et al., 2018). It was useful for CWs containing high phosphorus concentration wastewater in combination with a greenhouse structure at low temperatures (Etesami and Maheshwari, 2018).

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Fig. 4 "Plant-benthos-microbe" synergistic enhancement type CW.

4.3. Combination or integration with constructed wetlands

In addition to general pollutant removal by CWs, recently several treatment processes involving the degradation and transformation of specific pollutants by CWs in combination or integrated with systems such as algae ponds, photocatalytic oxidation processes, and metal redox processes have aroused broad attention. These combinations may improve the comprehensive treatment of pollutants in CWs compared to the application of a single technology.

# 4.3.1. Combination with algae ponds or photocatalytic oxidation

CWs in combination with stabilization ponds could improve secondary wastewater treatment, especially of dispersed rural wastewater or of concentrated wastewater in littoral zones at low temperatures (Šereš et al., 2017). In areas with many eco-ditches and channels, it was preferable to lower construction costs (Ham et al., 2004). Likewise, Zhao et al. (2016) significantly enhanced the TN removal of a CW combined with a high-rate algal pond through the oxygen enrichment of algae photosynthesis under 0-10°C. Meanwhile, the algae residue in CW may be used as carbon source by microbes in low carbon-nitrogen ratio wastewater. Aquaponics and greenhouse structures could also be applied to CWs (Lin et al., 2003). The ecological CW pond closely combined wastewater treatment with the comprehensive utilization of resources, which provided the economic benefits for local freshwater aquaculture and organic agriculture industries

(Hu et al., 2012; Wu et al., 2014).

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Photoelectrons and photogenerated holes generated by irradiation react with oxygen and oxides of wastewater in different positions of TiO2, translating them into OH and O<sub>2</sub> with strong oxidizing abilities (Antoniadis et al., 2007). The technology of TiO<sub>2</sub> photocatalysis for the degradation of several specific pollutants in wastewater has been shown in recent studies (Arana et al., 2008; Herrera-Melian et al., 2012; Wu et al., 2016). Interestingly, in combination with CWs, this system had a strong tolerance of peak hydraulic loads and achieved COD and total organic carbon removal efficiencies of 98.6% and 90%, respectively. The combination with CWs eliminated the deficiencies of the TiO<sub>2</sub>-photocatalysis process alone. Arana et al. (2008) achieved an 84% phenol degradation rate with TiO<sub>2</sub>-photocatalysis, but the concentration of the hydroquinone produced as an intermediate product was more poisonous than the phenol. The concentrations of both substances were decreased to satisfy the discharge standard through the additional effect of the CW. Likewise, solar TiO<sub>2</sub>-photocatalysis degraded the 4-nitrophenol with sunlight during the day and the CW, as a pretreatment, further treated the effluent at night; this treatment mode was deemed to be remarkably flexible and efficient (Herrera-Melian et al., 2012). It was worth mentioning that Palmqvist et al. (2015) demonstrated the feasibility of nanomaterials in natural purification systems by growing a fungal population in the rhizosphere of rapeseed and protecting the root from fungal infection using nano-TiO<sub>2</sub> particles. To utilize the strong photoreactivity of nano-TiO<sub>2</sub> (Legrini et al., 1993; Luan

et al., 2014), the nanocatalyst particles were loaded onto the surface of the nanofiller with a structure designed to induce micropollutants to form a catalytic active center through modification (Luan et al., 2014). With the pollutants adsorbed to the surface of the carrier, the photocatalytic oxidation technology was applied to industrial wastewater (Abdelbasir and Shalan, 2019). This nanomaterials-photocatalysis in situ treatment system could adsorb more pollutants due to its specific surface area. Moreover, the drawbacks of CW treatment of high-strength wastewater and bio-refractory compounds such as POPs could be offset (Kundu and Mondal, 2019).

# 4.3.2. Integration with metal redox

Biogenic manganese (Mn) oxides with strong oxidizing properties and catalytic reactivity enhanced the removal of the emerging organic pollutant triclosan (TCS) and of common pollutants due to the recycling between the different valence states of Mn in anoxic and aerobic zones of CWs (Xie et al., 2018). In the anoxic zone, pollutants including TCS, NH<sub>4</sub>+-N, and organic carbon compounds were absorbed and then oxidized by Mn oxides (birnessite). In this process, Mn<sup>4+</sup> converts to Mn<sup>2+</sup>, and NO<sub>3</sub>-N was removed by denitrification coupled with Mn<sup>2+</sup> oxidation. Subsequently, in the aerobic zone, the accumulated Mn-oxidizing bacteria reoxidize Mn<sup>2+</sup> and produced biogenic Mn oxides with high specific surface area for the removal of low molecular products derived from the anoxic zone. Based on the circulation of the Mn valence state (Fig. 5), the removal efficiencies of TCS and NH<sub>4</sub>+-N in CW could be increased by

21.6% and 33.7% at a depth of 30 cm compared with those in a non-Mn CW. This use
of metal redox is a good idea with the potential to address typical emerging organic
pollutants and reduce the cost of substrates in CWs at low temperatures (Xie et al.,
2018).

Fig. 5 The mechanism of CW integrated with Mn oxides for common and emerging organic pollutant removal.

In general, there was a great potential for the wetland plants in the effectiveness of wastewater treatment at high latitudes as the improvement and development of this technology (Raju et al., 2018). The additional plant and benthos would consume the huge manpower and material resources, and their investment was a considered factor (Liu et al., 2017). In terms of the engineering applications and the recycling of this system (Luan et al., 2013), the problems of the ecological safety of nanoparticles and the cost of nanomaterials were still need to be further addressed (Tahir et al., 2019). From a security perspective, the concentration of residual Mn in wastewater must be monitored constantly (Xie et al., 2018).

# 5. Conclusions

To address the bottleneck of wastewater treatment in CWs at low temperatures,

countermeasures enhancing operational performance through direct or indirect
modifications have been carried out and have increased pollutant removal efficiency to
a certain degree. Based on the analysis of pollutant removal mechanisms and pathways
in CWs at low temperatures, in the long term, future directions for enhancing CW
treatment efficiency and sustainable operation could focus on several aspects, including
mutation breeding for obtaining cold-resistant plants, bio-manipulation for perfecting
CW ecosystems, and the combination or integration of CWs with technologies such as
algae ponds, photocatalytic oxidation, and metal redox.

# Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 51720105013, 51878388 and 51978385), National Science Fund for Distinguished Young Scholars (No. 51925803), Shandong Provincial Natural Science Foundation (No. ZR2018QEE006), and the Major Program of Shandong Province Natural Science Foundation (No. ZR2018ZC08N4).

# Appendix A. Supplementary data

E-supplementary data for this work can be found in e-version of this paper online.

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973	Figures caption
974	Fig. 1 The distribution of CWs in administrative regions of China (The site and size of
975	circles indicates provincial capital and area respectively).
976	Fig. 2 Main pathways of pollutants removal in CWs. (1) OP: Organophosphorus; DP:
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981	temperatures.
982	Fig. 4 "Plant-benthos-microbe" synergistic enhancement type CW.
983	Fig. 5 The mechanism of CW integrated with Mn oxides for common and emerging
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995	Table caption
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997	MI indicates microbial inoculant. WT means wastewater type including raw (R), town
998	(T), domestic (D), synthetic (S), farm (F), municipal (M) wastewater. T is the
999	temperature.
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1016	Supplementary file
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1018	Fig. S1 The area distribution of Ramsar sites around the world (Dixon et al., 2016;
1019	Davidson and Finlayson, 2018).
1020	New insights for enhancing the performance of constructed wetlands
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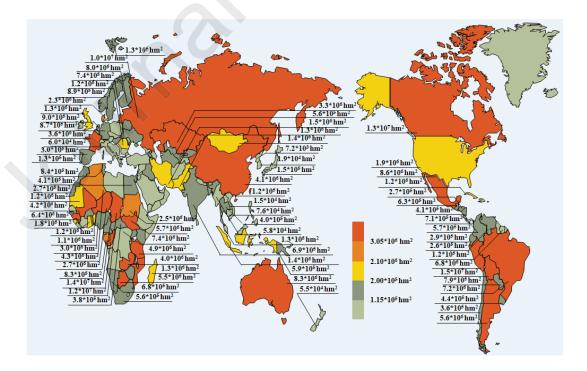


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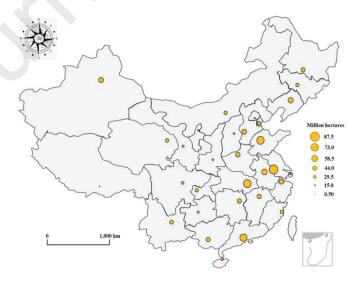
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### Figures caption

- Fig. 1. The distribution of CWs in administrative regions of China (The site and size of
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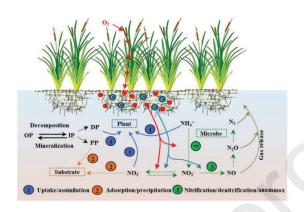


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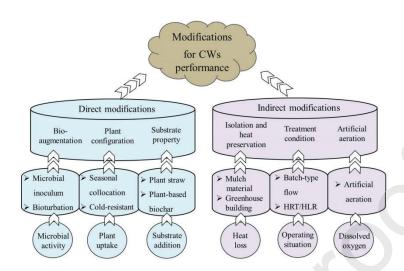


Fig. 3. Modifications were for responding to the cold stress on CW performance at low

1075 temperatures.

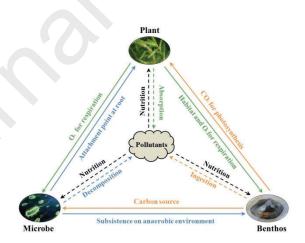


Fig. 4. "Plant-benthos-microbe" synergistic enhancement type CW.



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Fig. 5. The mechanism of CW integrated with Mn oxides for common and emerging

1081 organic pollutant removal.

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## Table caption

Table 1 Modifications were for enhancing CWs performance at low temperatures.

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Table 1 Modifications were for enhancing CWs performance at low temperatures.

Modificati on	Method	CW type	Scale	W	HRT	T (°C)	Removal efficiency (%)				Reference
							COD	NH <sub>4</sub> <sup>+</sup> -N	TN	TP	_ Kererenee
	MI	VSSF	Lab	R	4 d	8-12	60	84.5	74.2	NA	(Zhao et al., 2016)
Bio-aug- mentation	MI	FSS	Pilot	T	3 d	5	30	35.4	33.6	28	(Duan et al., 2016)
memation	MI	HSSF	Lab	D	4 d	7-11	78.5	83.1	74.6	68	(Faulwette r et al., 2009)

											(Chong
	MI	HSSF	Lab	S	1 d	10	47.3	31.9	56.1	NA	et al.,
											2012)
	Benthos	HSSF	Lab	S	13 d	1.6	NA	56.9	05 1	NIA	(Kang et
	Dellulos	HSSF	Lab	S	13 u	1-6	ΝA	36.9	85.1	NA	al., 2016)
	Batch	VSSF	т 1	D	24 h-	5-10	81.1	85.6	48.4	NA	(Zhou et
Operating			Lab		12 h						al., 2007)
condition	Batch	HSSF	Pilot	D	12 h-	8-10	95.1	74.4	78.7	NA	(Zhang et
					12 h						al., 2012)
	Hybrid				NA	6.4	77.7	78.2	85.5	74.9	(Harada et
	CW	-	Full	F							al., 2015)
	Hybrid			M	401	1-17	88-91	92-98	51.4	67-77	
		-	Pilot		13 h-						(Wang et
Integra-	CW				33 h						al., 2017)
tion	Green-	HSSF	Full	D	4 h-	2-5	85	70.98	NA	36.5	(Gao and
	house				35 h						Hu, 2012)
											(Zhao et
	Algal pond	VSSF	Lab	S	5 d	5-10	70.3	NA	81	71.9	al., 2016)

MI indicates microbial inoculant. W means wastewater type including raw (R), town (T),

domestic (D), synthetic (S), farm (F), municipal (M) wastewater. T is the temperature.

**Declarations** 

No conflict of interest, informed consent, human or animal rights were applied.

- Microbial activity is main restrictive factor on CW operation at low temperatures.
- Mutation breeding and nanomaterial provide a choice for CW sustainable development.
- CW combined other treatment processes to remove specific pollutants is a trend.