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Review

Bioaugmentation of treatment wetlands – A review

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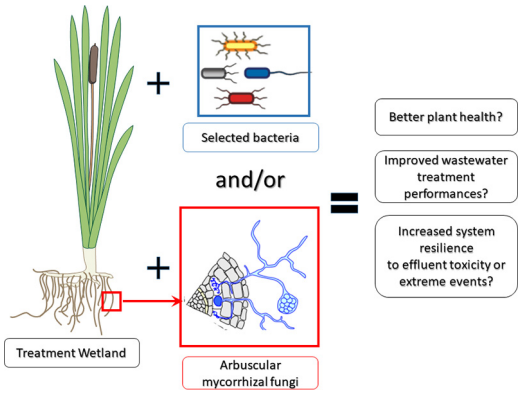
HIGHLIGHTS

- Overview on research publications evaluating bioaugmentation of treatment wetlands
- Artificial mycorrhization of plant roots and bacterial inoculation were included.
- Investigations were mostly performed on small scale/laboratory scale.
- Wetland plant growth positively effected by inoculation with arbuscular mycorrhiza.
- Bacterial inoculation tended to promote plant growth and pollutant degradation.

GRAPHICAL ABSTRACT



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ABSTRACT

Bioaugmentation in the form of artificial mycorrhization of plant roots and bacterial inoculation has been successfully implemented in several fields including soil remediation or activated sludge treatment. Likewise, bioaugmentation seems a promising approach to improve the functioning of treatment wetlands, considering that natural mycorrhization has been detected in treatment wetlands and that bacteria are the main driver of contaminant degradation processes. However, to date, full scale implementation seems to be rare. This review synthesizes the effects of bioaugmentation on different types of treatment wetlands, to a large extent performed on a microcosm (<0.5 m^2) or mesocosm scale (0.51 to 5 m^2). While inoculation with arbuscular mycorrhizal fungi tended to show a positive effect on the growth of some wetland plants (e.g. *Phragmites australis*), the mechanisms underlying such positive effects are not well understood and the effects of upscaling to full scale treatment wetlands remain unknown. Bacterial inoculation tended to promote plant growth and pollutant degradation, but longer term data is required.

Contents

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1. Introduction

In soil remediation and wastewater treatment, bioaugmentation usually refers to the process of inoculating a polluted environment (contaminated soil, sludge, water) with natural or genetically modified microorganisms that have the desired catalytic capabilities to remove some target organic contaminants (Hairston et al., 1997; Kuiper et al., 2004; Herrero and Stuckey, 2015). This green technology has been successfully used to treat activated sludge (Soda et al., 1998), industrial wastewater (Raper et al., 2018), soil contaminated with pesticides (Cycoń et al., 2017) or petroleum hydrocarbon products (Zaida and Piakong, 2018). Alternatively, other projects have focused on the use of plants to solve environmental issues through phytoremediation (Shmaefsky, 2020). The latter capitalizes on plant capacities for extracting or stabilizing substrate contaminants, or promoting their degradation in their rhizosphere.

Phytoremediation and bioaugmentation can be used in combination to improve treatment performance compared to either approach alone. Added microorganisms may promote plant performance by enhancing nutrient availability, and reducing plant stress response to contaminants, or act as biocontrol agents against native pathogens present in the growth substrate (Lebeau et al., 2008). They may also promote plant contaminant uptake through the production of solubilizing agents (e.g., siderophores, organic acids) or promote rhizodegradation of organic contaminants by secreting biosurfactants (Wang et al., 2017). Symbiotic arbuscular mycorrhizal (AM) fungi have specifically been shown to improve plant growth and trace element uptake in phytoremediation experiments (e.g., Turnau et al., 2005). Conversely, plants may promote biodegradation of organic contaminants by stimulating microbial biomass and activity. The plant rhizosphere is a well-known hotspot of microbial activity (Kuzyakov and Blagodatskaya, 2015) and the labile, root-derived carbon (C) contributes to microbially driven rhizodegradation of contaminants by improving soil structure and aeration, and enhancing desorption of organic pollutants from organic matter and colloids (Juhanson et al., 2009).

Treatment wetlands (TWs) can be considered as a specific case of phytoremediation. In TWs, effluents are treated through the combined actions of filtration, microbial degradation processes, adsorption and precipitation. Plants in TWs play several important roles: they provide attachment sites for microbial biofilms, release oxygen and organic compounds that stimulate microbial activity, remove nutrients by plant uptake and diffuse liquid flow (i.e. limit what has been coined hydraulic "short-circuiting" (Min and Wise, 2009)), thus improving treatment efficiency. Just like terrestrial plants, wetland plants are extensively colonized by microbial epiphytes and endophytes (Li et al., 2010; Clay et al., 2016). Similar avenues could thus be considered in TWs, whereby the inoculation of these phytoremediation systems could improve their overall efficiency. Yet, bioaugmentation in TWs has not attracted as much attention as bioaugmentation in soil remediation or conventional wastewater treatment. To our knowledge, only one study has reported the bacterial inoculation of a TW on a large scale (Austin et al., 2019). Some studies have shown that TW plants can be colonized by AM fungi (e.g., Stenlund and Charvat, 1994; Mantovi et al., 2002; Fester, 2013; Calheiros et al., 2019), and Zhang et al. (2011) have suggested potential implications of these symbionts to increase substrate enzymatic activities (promoting bacterial-derived or plant-derived enzymes). However, little evidence exists to show that bioaugmentation of TWs through bacterial or AM fungal inoculation substantially improves their efficiency (Chagnon and Brisson, 2017; de-Bashan et al., 2012). The present review aims to close this gap by synthesizing the available data from peer-reviewed studies on bioaugmentation of TWs. A structured literature search was performed in order to determine whether (i) bioaugmentation by AM fungal or bacterial inoculation has a positive effect on plants in TWs in terms of growth, resistance towards pollutants or "plant health" (photosynthetic rate, etc.) and whether ii) bioaugmentation improves pollutant removal in TWs.

2. Literature review

Specific combinations of keywords were chosen for a search in webofknowledge.com and sciencedirect.com for studies on both artificial mycorrhization and bacterial inoculation of TWs, the results of which are succinctly summarized in Table S1 (supplementary material). The search was continuously updated; the most recent update was performed on June 30, 2020. Only original research articles presenting studies mimicking TWs were considered, including:

- experimental setup constructed and operated according to a specific type of TW;
- pot studies of plants grown in an engineered substrate, or
- simulating free-surface flow wetlands using plants grown hydroponically.

In addition, to be considered, the experimental conditions of the studies had to be clearly described, e.g. the size of the treatment units, duration and analytical procedures. Publications with setups for bioremediation of soils were not included.

2.1. Mycorrhization

The search with the keywords yielded a total of 1520 publications on mycorrhization. Table S1 (supplementary material) summarizes the specific search match results. After removing duplicates, a total of 1237 publications remained. The titles of all remaining studies were then screened according to their relevance to the topic, and, in case of a positive outcome, the abstract and full text were consulted. Additional screening was conducted to eliminate irrelevant research.

Twenty publications dealt with artifical mycorrhization of wetland plants in a controlled environment (Fig. S1, supplementary material), of which three studies had to be excluded from further evaluation since crucial information was lacking (e.g. size of the experimental setups). One study found insufficient mycorrhization of the plants and did not investigate the results further (Wetzel and van der Valk, 1998). In the end, 17 publications met our criteria.

2.2. Bacterial inoculation

The combination of keywords resulted in a total of 793 publications on bacterial inoculation. Table S1 (supplementary material) summarizes the specific search match results. After removing duplicates, a total of 705 publications remained and were reviewed in the same way as those on mycorrhization. Studies using an unspecified inoculant such as sludge, or in which information on the bacterial strains was not provided, were not considered further. Research papers dealing with the investigation of microbial communities in natural and treatment wetlands, or those on the use of bioaugmentation in soil remediation or other wastewater treatment processes, were excluded from further evaluation.

Thirty-eight publications dealt with bacterial inoculation of TW systems in a controlled environment (Fig. S1, supplementary material), of which 13 were excluded from further evaluation since crucial information was lacking (e.g. size of the experimental setups).

3. Experimental settings

3.1. Mycorrhization

The 17 published articles represented 15 different studies, including two for which results were split for publication in different journals (Table 1). We recorded the type of response variables characterized in each study (e.g., plant health indicators, pollutant removal efficiency, etc.). All experiments were performed in greenhouses or growth chambers using either natural light, natural light with supplemental lighting or completely artificial light. Studies were thus conducted somewhat independently of local climate conditions. Temperatures and photoperiods were provided for most studies. However, humidity measurements in greenhouses was generally not mentioned.

From one to three plant species were tested in each study, combined with usually one type of mycorrhizal treatment, either with a single species innoculant or a mixture of different AM fungal strains. All studies also included replicates and non-mycorrhizal controls, but none used unplanted controls. Only two studies were on a mesocosm scale (Gao et al., 2020; Lingua et al., 2015; see Table 1), while all others described microcosm experiments, mostly in pots with a diameter of 9 to 21 cm and a height of 8 to 15 cm. All studies used a growing substrate, with the exception of Gao et al. (2020), which simulated a floating treatment wetland (FTW) setup. Study durations were between 21 and 100 days (Fig. 1). All studies except Gao et al. (2020) used drinking or deionized water, some of them spiked with nutrient solutions. Additionally, the impact of heavy metals was evaluated in three studies (Huang et al., 2017a, 2017b, 2018; Wang et al., 2017; Xu et al., 2019). Gao et al. (2020) evaluated the impact of AM on the treatment of low-saline wastewater.

Phragmites australis was the most frequently investigated plant, used in six studies, with three different types of AM fungal inocula: *Rhizophagus irregularis* in two microcosm studies (Huang et al., 2017a, 2017b, 2018; Wang et al., 2017), *Funneliformis mosseae* in one microcosm (Xu et al., 2019) and one mesocosm study (Lingua et al., 2015), and two studies using an unspecified mixture of AM fungal strains (Liang et al., 2018, 2019). *Lythrum salicaria* was used in three studies, and *Panicum hemitomon* in two. Both were always inoculated with AM fungal mixtures. The total number of macrophyte species covered is 13 (Fig. 2), whereas the number of AM fungal species is not clear due to the unspecified or only partially specified mixtures used in nine studies. Among those, six used a root based inoculum harvested from naturally mycorrhizal plants or soil (Stevens and Peterson, 2007; Stevens et al., 2002, 2011; Liang et al., 2018, 2019; Ipsilantis and Sylvia, 2007), and one identified only the genus level (Fraser and Feinstein, 2005).

Mycorrhizal inoculation success was reported in ten out of fifteen studies as the root mycorrhizal colonization frequency, which was determined through root staining with different dyes: trypan blue (Fraser and Feinstein, 2005; Xu et al., 2019; Hu et al., 2020; Ipsilantis and Sylvia, 2007; Gao et al., 2020), acid fuchsin (White and Charvat, 1999; Tang et al., 2001), chlorazol black E (Stevens and Peterson, 2007; Stevens et al., 2002) or methyl blue (Lingua et al., 2015) (cited methods: Phillips and Hayman, 1970; Kormanik and McGraw, 1982; Brundrett et al., 1984; Norris et al., 1994). The most commonly used method to evaluate the percentage of colonization was the gridline intersect method optimized by McGonigle et al. (1990) (Fraser and Feinstein, 2005; Stevens and Peterson, 2007; Stevens et al., 2002; White and Charvat, 1999; Ipsilantis and Sylvia, 2007) or in an earlier version by Giovannetti and Mosse (1980) (Tang et al., 2001; Gao et al., 2020). Two studies (Hu et al., 2020; Lingua et al., 2015) used the method proposed by Trouvelot et al. (1986), which also takes into account the intensity of colonization, as opposed to intersect methods, which only record fungal presence in or absence from root sections. One study (Xu et al., 2019) did not describe the method used to evaluate root mycorrhizal colonization.

All studies except Gao et al. (2020) investigated the effect of AM inoculation on biomass development, i.e. recorded data on at least one indicator of plant biomass, whether root, shoot, stem, leaves and/or total biomass (in most cases using dry biomass). For the evaluation of biomass, the only results considered here were those that did not include other effects such as resistance to pollution (e.g. heavy metals); however, different water regimes (different drying-wetting cycles or waterlogging) were in some cases part of the study design and were therefore described accordingly. Other investigated topics were the effect of AM inoculation on plant nutrient accumulation, resistance to toxins and nutrient removal from treated water.

3.2. Bacterial inoculation

Twenty-four studies in 25 publications matched the criteria, most of them (75%) published over the last 5 years (Table 2). The study durations varied widely, ranging from 24 h to 2 years (Fig. 1). The majority of trials were conducted outdoors (15). In five studies, the location (outdoors or indoors) was not specified, and another five studies were under controlled conditions in greenhouses or laboratories. From one to four plant species were tested in each study, either treated with a single bacterial strain inoculant or a mixture of different strains/species. As a whole, in these 25 studies, 10 different macrophyte species and 21 different bacterial geni were tested (Fig. 3). *Bacillus* was most commonly used (12 studies), with *Pseudomonas* (9 studies) and *Acinetobacter* (six

Table 1
Studies describing the effect of mycorrhization on treatment wetland performance in greenhouses/laboratory environment.

Stu-dy #	Authors	Plants	Substrate (sterilized yes/no)	Location	System size	Influent	Repli-cates[a]	Study duration (days)	Mycorrhizae
1	Hu et al. (2020)	*Phalaris arundinacea; Scirpus sylvaticus*	Sand 0.1–0.5 mm (yes)	Czech Republic	micro	1/4-strength Hoagland solution (Hoagland and Arnon, 1938) with ~1787 g $P \cdot m^{-2} \cdot y^{-1}$	5	~91	*Rhizophagus irregularis*
2	Gao et al. (2020)	*Canna indica; Cyperus alternifolius*	None (FTW)	China	*meso*	Domestic WW + salt	1	~30	Unspecified mix
3	Xu et al. (2019)	*Phragmites australis*	Vermiculite:sand mix 1:1 with four different TiO_2NP concentrations added (yes)	China	micro	modified Hoagland nutrient solution (0.22 mg $P \cdot L^{-1}$)	3	60	*Funneliformis mosseae*
4	Liang et al. (2019)	*Phragmites australis*	Soil:sand mix 1:1 0–2 mm (yes) +/− biochar 1%	China	micro	Distilled water	8	75	Unspecified mix
5	Liang et al. (2018)	*Phragmites australis*	Soil:sand mix 1:1 0–2 mm (yes)	China	micro	Nutrient solution (~21.1 g $P \cdot m^{-2} \cdot y^{-1}$)	8	64	Unspecified mix
6	Huang et al. (2017a, 2017b, 2018)	*Phragmites australis*	Vermiculite (yes)	China	micro	DW + Cd	2 / 3 / 3	21	*Rhizophagus irregularis*
7	Wang et al. (2017)	*Phragmites australis*	Vermiculite (yes)	China	micro	DW + Cd	3	21	*Rhizophagus irregularis*
8	Lingua et al. (2015)	*Phragmites australis*	Tanks filled to 1/3 with cobbles (8–15 mm)	Italy	meso	DW with 0, 30, 90 and 270 mg L^{-1} KNO_3	4	46	*Funneliformis mosseae*
9	Stevens et al. (2011)	*Bidens frondosa; Eclipta prostrata*	Masonry sand (no)	USA	micro	1/64 Long Ashton nutrient solution (Hewitt, 1966) (~17.5 g $P \cdot m^{-2} \cdot y^{-1}$)	3	50	Unspecified mix
10	Stevens and Peterson (2007)	*Lythrum salicaria*	Sand (no)	USA	micro	DW	10	50	Unspecified mix
11	Ipsilantis and Sylvia (2007)	*Panicum hemitomon; Typha latifolia*	1:1:1 mix peat: vermiculite:sandy, low-P, low-organic matter soil with 0, 20, and 50 mg kg^{-1} P added (yes)	USA	micro	DW	5	~100	Unspecified mix
12	Fraser and Feinstein (2005)	*Carex tribuloides; Phalaris arundinacea; Rumex orbiculatus*	Sand (yes)	USA	micro	DW	3	42	Mix (incl. *Glomus* spp., *Gigaspora* spp.)
13	Stevens et al. (2002)	*Lythrum salicaria*	Sand (no)	USA	micro	1/5 Long Ashton nutrient solution with P-concentrations from 0 to 40 mg $\cdot L^{-1}$	10	56	Unspecified mix
14	Tang et al. (2001)	*Typha angustifolia*	Silica sand (yes)	USA	micro	modified half-strength Hoagland's solution with P-concentrations from ~6 to 3090 g $\cdot m^{-2} \cdot a^{-1}$	5	91	*Funneliformis mosseae* (syn. *G. mosseae*)
15	White and Charvat (1999)	*Lythrum salicaria*	2:1 mix of No. 40:No. 20 sand (yes)	USA	micro	nutrient solution with P-concentrations from 0.03 to 15.5 mg $\cdot L^{-1}$	6	63	Mix (incl. *Glomus albidum, G. caledonium, G. etunicatum, G. microcarpum*)

DW: drinking water.
WW: wastewater.
[a] Replicates: number of units per species per treatment. One (1) means no replication.

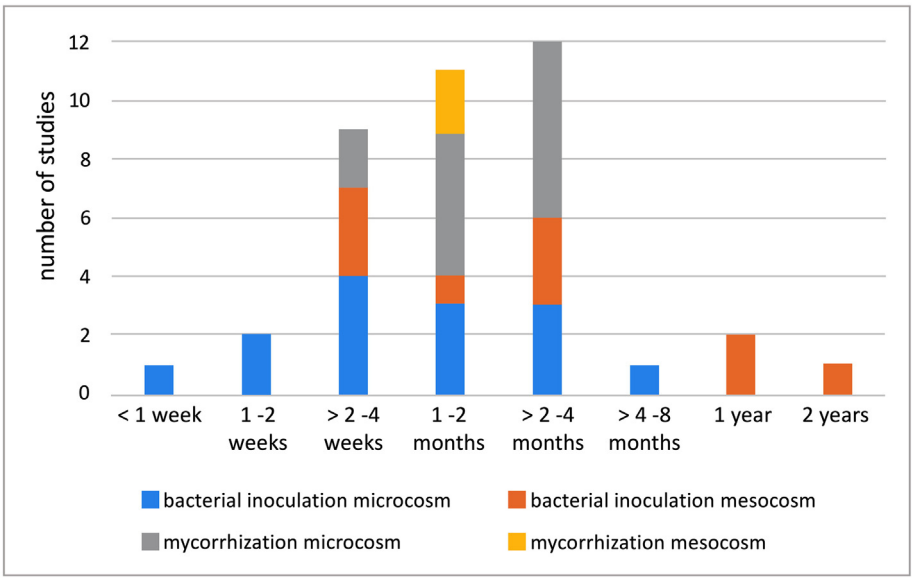

Fig. 1. Study duration for bacterial inoculation and mycorrhization.

studies) also frequently used. Bacterial strains tended to be selected according to their potentially beneficial properties, either from pre-trials or prior knowledge based on the literature. This included their potential to increase the removal of organic pollutants, nutrients and industrial chemicals, as well as to increase biomass growth and enhance plant resistance towards toxins (Fig. S2, supplementary material).

Fourteen studies were performed on a microcosm scale, ten on a mesocosm scale, and none on a large scale (>5m^2, Brisson and Chazarenc, 2009). Seven studies did not include replicates (Zhao et al., 2016, 2019; Shuai and Jaffe, 2019; Pei et al., 2016; Shao et al., 2013; Al-Baldawi et al., 2017; Ismail et al., 2020), whereas the others used 3 to 6 replicates. Nine studies used unplanted controls with bacterial inoculation (Nimkar et al., 2012; Shao et al., 2014; Ijaz et al., 2015; Saleem et al., 2019; Shahid et al., 2020; Rehman et al., 2018/2019; Fahid et al., 2020; Hussain et al., 2019; Tara et al., 2019). Two studies compared different types of TWs (Hussain et al., 2018b, 2019).

Two wetland types were each tested multiple times by the same research group: all six studies on FTWs (Ijaz et al., 2015; Saleem et al., 2019; Shahid et al., 2020; Rehman et al., 2018/2019; Fahid et al., 2020; Tara et al., 2019) were conducted in the same institution in Pakistan, and the three on submerged Vertical Flow Wetlands (VFWs) were from China (Shao et al., 2013, 2014, 2016).

The types of treated wastewater varied, but were most frequently domestic wastewater (either raw Nimkar et al., 2012; Shao et al., 2014, 2016; Zhao et al., 2016, 2019), pre-treated (Shuai and Jaffe, 2019), or mixed with industrial effluents (Ijaz et al., 2015)), or industrial wastewater (from oil and diesel production (Rehman et al., 2018/2019; Fahid et al., 2020), textile production (Nawaz et al., 2020; Hussain et al., 2019; Hussain et al., 2018a, 2018b, 2019; Tara et al., 2019) and tanning (Ashraf et al., 2018)). Several studies focused on the removal of spiked substances from drinking water or synthetic wastewater. Eight studies (Saleem et al., 2019; Ashraf et al., 2018; Rehman et al., 2018/2019; Fahid et al., 2020; Hussain et al., 2018a, 2018b, 2019) compared planted systems in the following way:

- inoculated systems treating wastewater;
- systems without inoculation treating wastewater; and
- systems without inoculation fed with drinking water.

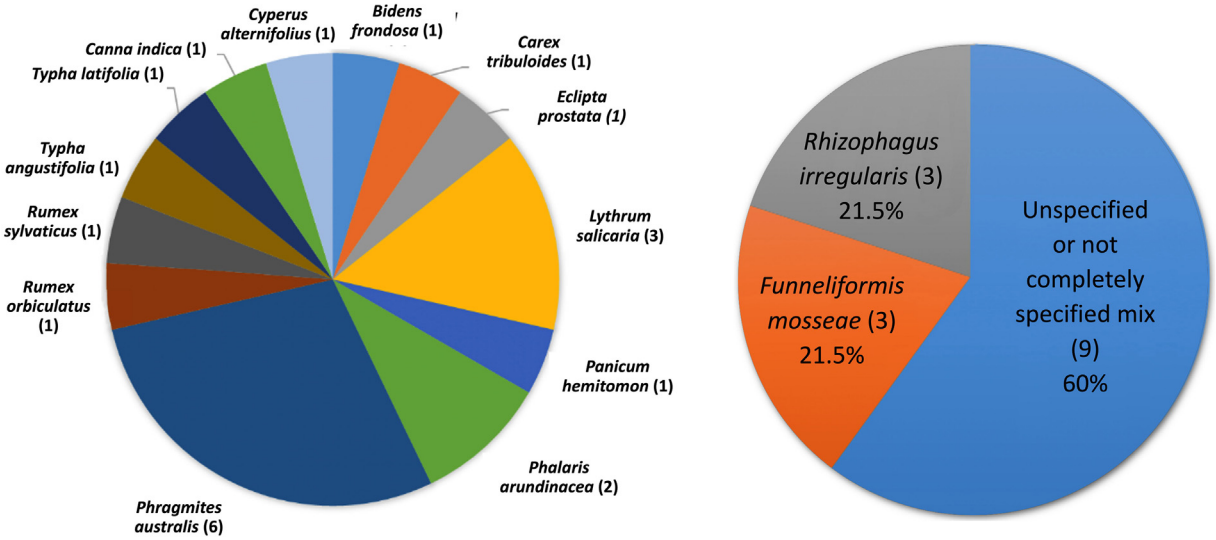

Fig. 2. Plant species investigated in 15 different studies on artificial mycorrhization (left) and mycorrhize species used for inoculation (right); total number of studies per species and percentage given.

Table 2
Studies describing the effect of bacterial inoculation on treatment wetland performance.

Study #	Authors	Wetland type	Plants	Location	System size	Influent	Repli-cates[a]	Study duration after inoculation	Rep. of inocu-lation[b]	Bacteria (mix) at genus level (or higher)
16	Shahid et al. (2020)	FTW	*Brachiara mutica, Typha domingensis, Phragmites australis, Leptochloa fusca*	Pakistan	micro	Domestic + industrial WW + heavy metals	3	35 days	1	*Aeromonas, Delftia, Pseudomonas, Bacillus, Rhodococcus*
17	Fahid et al. (2020)	FTW	*Phragmites australis*	Pakistan	micro	DW with diesel	3	90 days	1	*Bacillus, Acinetobacter*
18	Nawaz et al. (2020)	FTW	*Phragmites australis*	Pakistan	meso	Industrial WW (textile production)	3	20 days	1	*Rhodococcus, Pseudomonas, Acinetobacter*
19	Ismail et al. (2020)	HFW	*Scirpus grossus*	Malaysia	meso	Synthetic mining WW	1	102 days	1	*Bacillus, Brevibacterium*
20	Saleem et al. (2019)	FTW	*Phragmites australis*	Pakistan	micro	DW with phenol (500 mg l^{-1})	3	15 days	1	*Acinetobacter, Bacillus, Pseudomonas*
21	Fu et al. (2019)	VFW	*Kandelia candel*	China	micro	Synthetic WW	3	35 days	1	*Zobellella*
22	Zhao et al. (2019)	VFW	*Acorus calamus*[d]	China	micro	Synthetic WW with atrazine	1	65 days	3	*Pseudomonas, Arthrobacter*
23	Shuai and Jaffe (2019)	VFW-upflow	*Schoenoplectus acutus*[d]	USA	micro	DW with nutrients	1	8 months	2	*Acidimicrobiaceae*
24	Hussain et al. (2019)	VFW; HFW	*Phragmites australis*	Pakistan	meso	Industrial WW (textile production)	6	3 months	1	*Pantoea, Microbacterium, Bacillus*
25	Tara et al. (2019)	FTW	*Phragmites australis*	Pakistan	meso	Industrial WW (textile production)	3	2 years	9	*Acinetobacter, Pseudomonas, Rhodococcus*
26	Rehman et al. (2019); Rehman et al. (2018)	FTW	*Brachiara mutica; Phragmites australis; Typha domingensis*[d]*; Leptochloa fusca*	Pakistan	micro	Industrial WW (oil production)	3	42 days	1	*Bacillus, Acinetobacter, Klebsiella*
27	Ashraf et al. (2018)	VFW	*Brachiaria mutica*	Pakistan	micro	Industrial WW (tannery)	3	27 days	1	*Microbacte-rium, Pantoea, Enterobacter*
28	Hussain et al. (2018a)	VFW	*Brachiaria mutica*	Pakistan	meso	Industrial WW (textile production)	6	1 year	1	*Pantoea, Microbacterium, Bacillus*
29	Hussain et al. (2018b)	HFW	*Leptochloa fuchsa*	Pakistan	meso	Industrial WW (textile production)	6	1 year	1	*Pantoea, Microbacterium, Bacillus*
30	Al-Baldawi et al. (2017)	HFW[c]	*Scirpus grossus*[d]	Malaysia	meso	DW with diesel (0.25%)	1	63 days	1	*Bacillus*
31	Shao et al. (2016)	VFW submer-ged	*Phragmites australis*	China	micro	Domestic WW	3	25 days	5	*Paenibacillus*
32	Zhao et al. (2016)	VFW	*Acorus calamus*[d]	China	micro	Domestic WW	1	105 days	3	*Bacillus, Pseudomonas, Thermoacti-nomyces, Laceyella, Saccharo-polyspora*[c]
33	Pei et al. (2016)	HFW	*Phragmites australis*	China	meso	Domestic WW	1	17 days	1	*Paenibacillus*
34	Ijaz et al. (2015)	FTW	*Brachiaria mutica*	Pakistan	micro	Domestic + industrial WW	3	192 h (8 days)	3	*Bacillus, Acinetobacter*
35	Lingua et al. (2015)	VFW sub-merged	*Phragmites australis*	Italy	meso	DW with nutrients	4	46 days	1	*Pseudomonas*
36	Shao et al. (2014)	VFW submer-ged	*Phragmites australis*	China	micro	Synthetic polluted RW; synthetic WW	3	7 days	1	*Pseudomonas, Paenibacillus*
37	Zhao et al. (2014)	VFW	*Phragmites australis*	China	micro	Synthetic WW with endosulfan	3	20 days	3	*Serratia, Alcaligenes, Labrys*
38	Shao et al. (2013)	VFW submerged	*Phragmites australis*	China	meso	Domestic WW	1	27 days	1	*Pseudomonas*
39	Nimkar et al. (2012)	HFW	*Schoenoplectus validus; Bambusa vulgaris*	India	micro	Domestic WW	3	24 h	1	*Bacillus*

DW: drinking water; FTW: Floating Treatment Wetland; FWS: Free Water Surface Flow Wetland; HFW: Horizontal Flow Wetland; RW: river water; VFW: Vertical Flow Wetland; WW: wastewater.
[a] Replicates: number of units per species per treatment. One (1) means no replication.
[b] Repetition: number of injections of bacterial inoculant during the trial. One (1) means inoculation at one time point only (no repetition).
[c] Mix of heterotrophic nitrifying bacterium, autotrophic nitrifying bacteria and a commercially available complex agent BZT incl. the listed ones.
[d] Nomenclature adapted: *Typha angustata = Typha domingensis; Calamus = Acorus calamus; Scirpus acutus = Schoenoplectus acutus.*

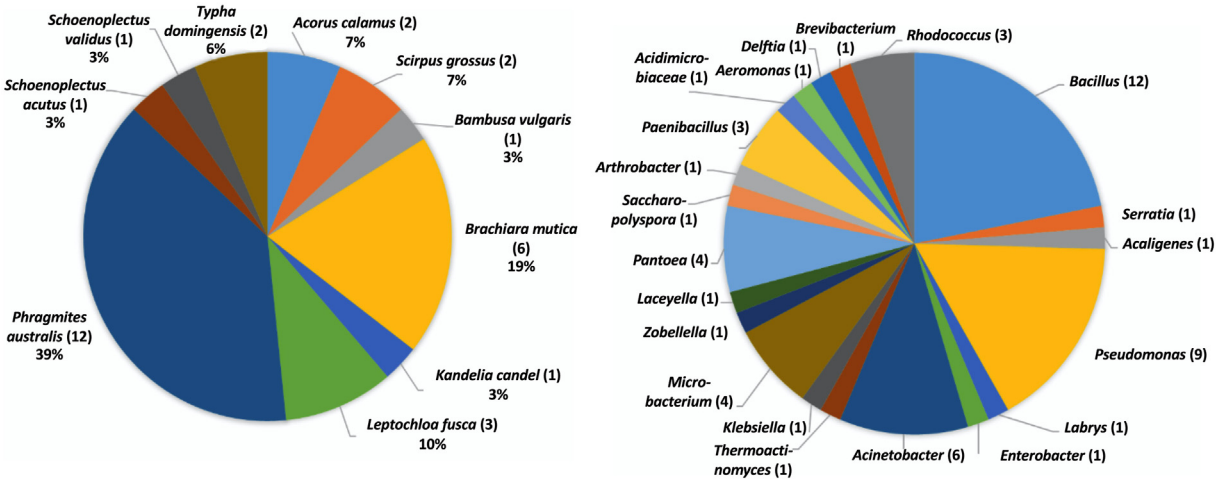

Fig. 3. Plant species investigated in 24 different studies on bacterial inoculation (left) and bacterial species at genus level used for inoculation in different mixes or as single inoculant (right).

This study design made it possible to investigate the impact of the inoculants on plant-growth inhibiting substances in the wastewater.

Table S2 (supplementary material) provides an overview of the combinations of plants and bacteria type at phylum level used in the studies. This highlights the fact that the only plant – bacteria combination repeated in three studies was *Phragmites australis* – *Proteobacteria*; six other combinations were tested in two studies each, while all other plant-bacteria combinations were used in single trials.

Seventeen studies investigated the success of bacterial inoculation by retrieving the bacterial strains from the rhizospheric water, the root and/or shoot tissues, the treated wastewater or from different substrate layers (Table S3, supplementary material). Methods used to investigate inoculation success included i) viable plate counting, and ii) DNA-based methods such as restriction fragment length polymorphism (RFLP), real time quantitative PCR (RT qPCR) or Sanger sequencing on specified regions such as intergenic spacers.

4. Effects of bioaugmentation

4.1. Mycorrhization

To date, the benefits of mycorrhization have not been tested under TW operating conditions and only in one study with wastewater as inflow.

4.1.1. Inoculation success

In the ten studies that investigated the inoculation success, eleven different plant species were tested: *Lythrum salicaria* in three studies, *Phragmites australis* and *Phalaris arundinacea* in two each; the eight other plant species only once. Additionally, studies tested different levels of nutrient addition and water availability to plants, which makes direct comparison difficult. For example, Ipsilantis and Sylvia (2007) compared the colonization of *Panicum hemitomon* and *Typha latifolia* between free drained and flooded conditions and under three different P-levels, whereas White and Charvat (1999) investigated *Lythrum salicaria* under five different P-levels under constantly saturated conditions. Despite these differences, general trends can be observed:

- Increasing concentration of P in the feed water had a negative impact on the percentage of mycorrhizal colonization: at very high levels (e.g. ≥10 mg $PO_4 \cdot L^{-1}$ in Stevens et al. (2002) and White and Charvat (1999)), colonization was totally supressed. The absence of additional P in the feed resulted in both highest mycorrhizal colonization compared to low dosage of P (Ipsilantis and Sylvia, 2007) and slightly lower colonization compared to low dosages of P (Stevens et al., 2002; White and Charvat, 1999).
- An increase in permanent water availability reduced colonization. The lowest values were found under flooding conditions, although this also depended on the plant species: in a study by Fraser and Feinstein (2005), *Phalaris arundinacea* showed the highest percentages of colonization in a flooded setting, compared to setups with lower water availability, while the colonization of *Carex tribuloides* responded negatively to higher water availability in the same study. However, the different N:P concentrations tested in the study might have also interfered with the results. Stevens et al. (2002) pointed out that the percentage of AM colonization in inundated or flooded soils is low and linked to the increased P availability under these conditions. This is partially reflected in the results of the different studies. Future studies should also investigate N:P stoichiometry specifically, as this has repeatedly been found to alter plant response to AM fungi (e.g., Johnson et al., 2003, 2015).

In the seven studies comparing the roots of the control group with the inoculated plants (Fraser and Feinstein, 2005; Stevens and Peterson, 2007; Xu et al., 2019; White and Charvat, 1999; Tang et al., 2001; Ipsilantis and Sylvia, 2007; Gao et al., 2020), the non-inoculated control plants showed no or very low percentages of colonization at the end of the study duration. The only exception is the study by Xu et al. (2019), which investigated the effect of mycorrhization on *Phragmites australis* with different substrate moisture content (50%, 70% and 100%) as well as different concentrations of TiO_2 nanoparticles (0, 100, 200, and 500 mg kg^{-1}). The highest rate of mycorrhization was reached for each substrate moisture condition for the microcosms not exposed to TiO_2 nanoparticles; while the control group reached a mycorrhization of up to 20%, the inoculated group reached up to 65%. An increase of TiO_2 nanoparticles reduced the colonization of both inoculated and control plants.

One study investigated the impact of salt stress on the mycorrhization of plant roots and found that a higher salt concentration in water can even lead to a higher degree of mycorrhization (Gao et al., 2020).

4.1.2. Biomass development

A positive impact of mycorrhizal inoculation on biomass development cannot be clearly determined based on the studies considered for this review. Also, only two plant species were tested in different studies in a way that the results were comparable:

- Five of the 6 studies that tested the effect of mycorrhizal inoculation on biomass development of *Phragmites australis* found a positive effect

on below-ground biomass (Table 3), independent of the AM fungal species used (*Rhizophagus irregularis, Funneliformis mosseae*, species mixture) and the duration of the experimental period, excluding inoculation (21 to 63 days) . On the other hand, only 3 out of 6 studies found a significant increase in shoot biomass and one study reported an increase in total plant biomass (out of the 4 studies that measured this variable). No study reported a decline in *Phragmites australis* biomass production following mycorrhizal inoculation. Because of the small number of studies reporting mycorrhizal colonization frequency in *Phragmites australis* roots (only 2), it is impossible to determine whether there is a general correlation between the amount of root colonization and the benefits derived from the symbiosis, as is the case for terrestrial (i.e., non wetland) systems (Treseder, 2013).

- The three studies investigating *Lythrum salicaria* did not find positive effects of mycorrhization for most investigated parameters. None of the studies on *Lythrum salicaria* could entirely explain why inoculation had almost no or even adverse effects, but the authors suggested that side effects of the experimental setups led to these results (White and Charvat, 1999), that mycorrhization could benefit plants on parameters not investigated in the experimental setup, e.g. increased pathogen or herbivore resistance or nutrient acquisition (Stevens and Peterson, 2007; Stevens et al., 2002), or that too short a study duration made it impossible to observe effects (White and Charvat, 1999). However, despite these claims, these results were in line with the low responsiveness of *Lythrum salicaria* to AM fungi (Philip et al., 2001), a tendency apparently shared among ruderal, exotic plants (e.g., Pendleton and Smith, 1983; Vogelsang and Bever, 2009).

All other plant species were only investigated in one study each. However, one impact on biomass development noted in three studies was water levels in the pots tested. Among these studies, two (Hu et al., 2020; Ipsilantis and Sylvia, 2007) showed a significantly larger biomass in inoculated plants when the water level was low, which was not the case with a higher water level. Stevens et al. (2011) did not show such a difference, possibly due to conditions such as those referred to above, where flooding can decrease colonization by AM, thereby reducing the differences between inoculated and control plants.

4.1.3. Resistance towards heavy metals

Other investigated effects were an increased resistance of mycorrhized plants towards heavy metals (Huang et al., 2017a, 2017b, 2018; Wang et al., 2017; Xu et al., 2019). In all studies, the inoculation of *Phragmites australis* with AM led to signifcantly greater root growth under heavy metal stress compared to the control group (25 to 130% higher depending on the Cd concentration added in Huang et al. (2017a), 27 to 113% higher depending on the TiO_2 nanoparticle concentration in Xu et al. (2019)). For the studies investigating the impact of cadmium, total biomass was only significantly higher over a given threshold (1 $mg \cdot L^{-1}$ for Wang et al. (2017), 2 $mg \cdot L^{-1}$ for Huang et al. (2017a)). Additionally, Xu et al. (2019) showed significantly higher N and P concentrations in the inoculated seedlings in the columns spiked with TiO_2 nanoparticles, as well as a higher water content, plant height, root length and other growth parameters. It also showed an increased concentration of TiO_2 in roots and a reduced concentration in leaves compared to non-inoculated plants, which indicates that AM inoculation suppresses pollutant transport. Huang et al. (2017a) showed the same effect for Cd.

Table 3
Results for biomass development (dry weight) with mycorrhizal inoculation organized by plant species.

Study #	Plant species	Inoculant	Study duration (days)	Substrate sterilized?	Seeds/roots sterilized?	Below-ground biomass (roots)	Above-ground biomass[a]	Total biomass
6	*Phragmites australis*	*Rhizophagus irregularis*	21	Yes	Yes	Myc > Non-Myc ($p < 0.01$)	Non-Myc > Myc for stem biomass ($p < 0.05$), no sig. difference for leaf biomass	no sig. diff.
7	*Phragmites australis*	*Rhizophagus irregularis*	21	Yes	Yes	Myc > Non-Myc ($p < 0.05$)	no sig. diff.	no sig. diff.
8	*Phragmites australis*	*Funneliformis mosseae*	46	No	No	Myc > Non-Myc ($p < 0.05$)	Myc > Non-Myc ($p < 0.05$)	–
3	*Phragmites australis*	*Funneliformis mosseae*	60	Yes	No	Myc > Non-Myc ($p < 0.05$) (fresh weight)	Myc > Non-Myc ($p < 0.05$) (fresh weight)	–
5	*Phragmites australis*	Unspecified mix	64	Yes	Yes	no sig. Diff.	depending on waterlogging conditions[b]	no sig. diff.
4	*Phragmites australis*	Unspecified mix	75	Yes	Yes	Myc > Non-Myc	no sig. diff.	Myc > Non-Myc
10	*Lythrum salicaria*	Unspecified mix	50	No	No	no sig. diff.	no sig. diff.	no sig. diff.
13	*Lythrum salicaria*	Unspecified mix	56	No	No	no sig. diff.	no sig. diff.	–
15	*Lythrum salicaria*	Mix	63	Yes	No	–	–	no sig. diff.
1	*Phalaris arundinacea*	*Rhizophagus irregularis*	91	Yes	Yes	no sig. diff. for water levels 5&9 cm below surface		–
			91	Yes	Yes	Myc > Non-Myc for water levels 9–11 cm below surface		–
9	*Bidens frondosa*	Unspecified mix	50	No	No	no sig. diff. regardless of water availability		
9	*Eclipta prostata*	Unspecified mix	50	No	No	no sig. diff. regardless of water availability		
11	*Panicum hemitomon*	Unspecified mix	100	Yes	Yes	Myc > Non-Myc	Myc > Non-Myc	–
1	*Scirpus sylvaticus*	*Rhizophagus irregularis*	91	Yes	Yes	no sig. diff. for water levels 5&9 cm below surface		–
						Myc > Non-Myc for water levels 9–11 cm below surface		–
14	*Typha angustifolia*	*Funneliformis mosseae*	91	Yes	Yes	–	Results inconclusive	–
11	*Typha latifolia*	Unspecified mix	100	Yes	Yes	no sig. diff.	no sig. diff.	–

- not investigated.
Myc: mycorrhized plants; Non-Myc: non-mycorrhized plants.
[a] At least one of the parameters shoots, stems or leaves.
[b] Three different drying-wetting cycles tested; only significant difference for drying-wetting cycle 2.

4.1.4. Treatment efficiency

Only one study (Gao et al., 2020) investigated the effect of mycorrhizal inoculation on the treatment efficiency in a FTW, in low salinity water. The study found positive effects of the FTW planted with mycorrhized *Phragmites* on the removal of total dissolved solids (TDS), chemical oxygen demand (COD), total phosphorus (TP) and total nitrogen (TN), but no statistical inferences were possible since the setup was not replicated.

4.2. Bacterial inoculation

4.2.1. Inoculation success

The overall results show that inoculation changed the microbial communities present in the substrate or water of the systems as well as those in the plant material. Five studies (Ijaz et al., 2015; Saleem et al., 2019; Ashraf et al., 2018; Rehmann et al., 2018/2019; Nawaz et al., 2020) compared the density of bacterial strains included in their inocula as measured in roots and shoots of the control vs. inoculated plants, and found higher densities in the inoculated plants, confirming the efficiency of the procedure. Fahid et al. (2020), Hussain et al. (2018a, 2018b, 2019) and Tara et al. (2019) looked only at the development in the inoculated systems and reported a high persistence, although some studies (e.g. Fahid et al., 2020) observed a decline in density of the inoculated strains over time. Studies comparing effluents from the inoculated/planted systems with those from the inoculated/unplanted systems (Ijaz et al., 2015; Saleem et al., 2019; Rehman et al., 2018/2019; Fahid et al., 2020; Hussain et al., 2018a, 2018b, 2019; Tara et al., 2019) predictably found that bacterial persistence was higher in planted replicates. Ijaz et al. (2015) and Fahid et al. (2020) investigated the water phase and found lower numbers of the inoculated bacteria in the effluent of the control groups compared to the planted control group; Nawaz et al. (2020) found a higher number of coliform units in the water of inoculated systems. Al-Baldawi et al. (2017) even found a significant correlation of the rhizobacteria population with time, biomass and root length. The positive effect of symbioses between rhizo- and endophytic bacteria and plants was generally invoked to explain these results (e.g. Weyens et al., 2009).

Three studies investigating the substrate revealed that bacterial diversity tended to be higher when experimental units were planted but not inoculated with exogenous bacterial strains. This was generally interpreted as evidence for competition between the microbial inocula and the resident bacteria present in the original substrates (Fu et al., 2019; Zhao et al., 2019; Zhao et al., 2016). One study found no difference in bacterial density between planted/non-inoculated, planted/inoculated and unplanted/inoculated systems (Ijaz et al., 2015).

Future studies should take advantage of high-throughput, next-generation sequencing techniques to track the development of inoculated strains' population sizes, as well as their relative competitive ability against resident taxa already colonizing the rhizosphere and the substrate. Most of the abovementioned studies used culture-based approaches or fingerprinting, which are known to have inherent biases such as failing to detect many unculturable taxa or lacking fine-scale resolution to discriminate related taxa (e.g., Lee et al., 2017). Knowing that competition between inoculants and resident taxa is a major factor shaping (1) success of establishment (Lottmann et al., 2000) and (2) ecological functions performed by the microbiome (Mallon et al., 2018), it is crucial to pay more attention to interspecific interactions involving inoculants.

4.2.2. Biomass development and resistance towards plant-growth inhibiting factors of wastewater

In general, inoculated systems had significantly greater plant biomass development than non-inoculated systems (Table 4). Five of the ten studies looking at biomass development were conducted on FTWs.

The studies were all fed with different kinds of wastewater, which in general had a negative impact on plant development compared to setups fed with drinking water (in six studies; the exception: the study by Ashraf et al. (2018)). However, comparison of plants grown in drinking water, control group and inoculated systems in industrial wastewater showed that bacterial inoculation can reverse the negative effects of potential toxins such as heavy metals or pesticides compared to non-inoculated control systems (Saleem et al. (2019) and Fahid et al. (2020)) for root and shoot biomass; root length in Fahid et al. (2020); shoot length and, partially, shoot biomass in Nawaz et al. (2020)).

Some studies looked at the effect of inoculation on the uptake of toxins such as heavy metals into the plant material. Although study Ashraf et al. (2018) did not show such a difference in the removal of heavy metals from tannery wastewater, the uptake in both roots and shoots of *B. mutica* was significantly higher in the inoculated systems for almost all of the 9 tested metals. The same was the case in Tara et al. (2019), where inoculation increased the metal uptake of *Phragmites australis*. A review on phytoremediation of metals by Sessitsch et al. (2013) suggests that the bacteria make trace elements more bioavailable.

4.2.3. Treatment efficiency

Bacterial inoculation tended to increase TW efficiency, irrespective of plant type, investigated parameter, bacteria used for inoculation, TW type, study duration or TW feed (Table 5). The sampling interval also played an important role in the removal efficiency measured: In several studies, samples were collected at different intervals following one feeding. This shows the development of the treatment efficiency during a single batch-flow in flow-through microcosms designed as VFW or HFW (Hussain et al., 2018a, 2018b, 2019), and it provides an estimate of the required hydraulic retention time in FTW microcosms (Ijaz et al., 2015; Rehman et al., 2018/2019; Fahid et al., 2020).

The hypothesized impact of the inoculated bacteria, as shown in Fig. S2 (supplementary material), can be both direct and indirect. Direct effects can be, for example, the uptake of ammonical nitrogen (NH_4-N) (Nimkar et al., 2012) or the degradation of hydrocarbons (Pal et al., 2016). Most of the effects observed are, however, likely to be indirect. Hussain et al. (2018a) observed an increase of oxygen availability after inoculation, which reduced chemical and biological oxygen demand (COD and BOD) by promoting decomposition of organic compounds. Likewise, the CO_2 production of inoculated heterotrophic bacteria can create a C substrate for chemoautotrophic nitrifiers, which leads to the consumption of NH_4-N as an energy substrate (Nimkar et al., 2012).

The interaction between inoculated bacteria and wetland plants plays a considerable role in improving removal efficiency, either by promoting plant growth and thus plant-derived services (e.g. Fahid et al., 2020; Tara et al., 2019), or by enhancing the bioavailability of pollutants such as heavy metals, which can promote extraction by plants (Sessitsch et al., 2013). Nawaz et al. (2020) found significant differences in the removal of heavy metals from industrial wastewater (textile production) in inoculated FTWs planted with *Phragmites australis*, compared to uninoculated controls. The authors attribute this to the bioaccumulation potential of the inoculated bacteria.

Various parameters can have an impact on the success of the inoculation, e.g. study duration and time elapsed since the last inoculation. Zhao et al. (2016, 2019) investigated the development of the inoculation effect over time in the same setup using different kinds of inoculant. While VFW efficiency (as measured by drops in COD, NH_4-N and TN) improved in the short term, VFW were indistinguishable from controls after 16 days (Zhao et al., 2016). Zhao et al. (2019) observed a similar drop in the impact of bacterial inoculation over time, although the inoculated TW always remained more efficient than uninoculated controls. Likewise, study duration would be an important parameter to consider in future studies, as initial samplings early in experimental studies do not always reveal significant differences between treatments and controls (e.g., Shahid et al., 2020). This could be because bacterial inoculants need to establish in the TW before they are able to influence its efficiency (but see Fahid et al., 2020). Collectively, these results show the

Table 4
Results for biomass development with bacterial inoculation organized by plant species. If not further specified, results refer to fresh and dry biomass.

Study #	Plant (TW type)	Study duration after ino-culation	Feed	Root biomass		Shoot biomass		Root length		Shoot length	
				Tap water treatment > other treatments	Inoculated > C	Tap water treatment > other treatments	Inoculated > C	Tap water treatment > other treatments	Inoculated > C	Tap water treatment > other treatments	Inoculated > C
20	*P. australis* (FTW)	15 days	DW spiked with phenol	++ > C; + > inoculated	fw: ++ dw: +	++ (> C)	++				
18	*P. australis* (FTW)	20 days	Textile bleaching WW[a]	dw: ++	dw: ++	dw: ++ (> C) ++ (> inoculated for one dye conc.)	dw: ++ for two dye conc.	++	++	++ (> C)	++ (> for two dye conc.)
16	*P. australis* (FTW)	35 days	Domestic + industrial WW spiked with heavy metals		dw: ++		dw: ++				
26	*P. australis* (FTW)	42 days	Industrial WW (oil production)	++	++	dw: ++ fw: ++ > C; + > inoculated	++	++ > C; + > inoculated	++	++	++
35	*P. australis* (VFW sub-merged)	46 days	Tap water with diff. nutrient concentrations		fw: only ++ for 90 $mg \cdot L^{-1}$ KNO_3 dw: ++ for all concentrations		only ++. for 0 $mg \cdot L^{-1}$ KNO_3				only ++ for 0 $mg \cdot L^{-1}$ KNO_3
17	*P. australis* (FTW)	90 days	Diesel contaminated water	fw: ++ > C; + > inoculated dw: + > C; − < inoculated treatment)	++	++ > C; + > inoculated	++	++	++	++	++
24	*P. australis* (VFW)	3 mths	Textile bleaching WW	dw: ++	dw: ++	dw: ++	dw: ++	dw: ++	++	++	++
24	*P. australis* (HFW)	3 mths	Textile bleaching WW	dw: ++	dw: ++	dw: ++	dw: ++	++	++	+	+
25	*P. australis* (FTW)	2 years	Industrial WW (textile production)	++	++	++	++	++	++	++	++
27	*B. mutica* (VFW)	27 days	Tannery WW	–	fw: ++ dw: +	–	++	–	+	–	++
16	*B. mutica* (FTW)	35 days	Domestic + industrial WW spiked with heavy metals		dw: ++		dw: ++				
26	*B. mutica* (FTW)	42 days	Industrial WW (oil production)	++	++	++	++	++	++	++	++
16	*T. domin-gensis* (FTW)	35 days	Domestic + industrial WW spiked with heavy metals		dw: ++		dw: ++				
26	*T. domin-gensis* (FTW)	42 days	Industrial WW (oil production)	++	++	++	++	++	++	++	++
16	*L. fusca* (FTW)	35 days	Domestic + industrial WW spiked with heavy metals		dw: ++		dw: ++				
26	*L. fusca* (FTW)	42 days	Industrial WW (oil production)	++	+	++	fw: + dw: ++	++	+	++	++
29	*L. fusca* (HFW)	1 year	Industrial WW (textile production)	++	++	++	++	++	++	++	++
19	*Scirpus grossus* (HFW)	102 days	Synthetic mining WW	investigated total plant weight, no distinction between roots and shoots; total plant dw: inoculated + > C; fw: C + > inoculated (no comparison with drinking water)							+

++: biomass is significantly higher; +: biomass is higher, but not significantly; −: biomass is lower, but not significantly; −-: biomass is significantly lower; dw: dry weight; fw: fresh weight; WW: wastewater; C: control (planted, non-inoculated)

Table 5
Differences in treatment efficiency between control TW and TW inoculated with bacteria (organized by type of TW).

Stu-dy #	Plant	Study duration after ino-culation	Feed	TW type	Removal efficiency in inoculated systems > non-inoculated systems								
					COD	BOD	TOC	TN	TP	TSS	TDS	Heavy metals	other
36	*P. australis*	7 days	Synthetic polluted RW	VFW sub-merged	+			+ (also for NH_4-N)	+				++ for phenol
36	*P. australis*	7 days	Synthetic WW	VFW sub-merged	+			+ (also for NH_4-N)	+				++ for phenol
24	*P. australis*	3 months	Textile bleaching WW	VFW	++	++ after 72 h	++	++	++	++ after 48 h	++	++ (Fe, Ni, Cd)	
27	*B. mutica*	27 days	Tannery WW	VFW	++	++		++	++ for PO_4-P	++		+	++ for sulfate, chloride and sodium; -- for oil and grease
28	*B. mutica*	1 year	Industrial WW (textile production)	VFW	+	= after 48 h		++ after 48 h	+		- after 48 h	++ for Cr, Fe, Ni	++ for color·m^{-1}
39	*S. validus*	24 h	Domestic WW	HFW		++		NH_4-N: ++ NO_3-N: +					
39	*B. vulgaris*	24 h	Domestic WW	HFW		++		NH_4-N, NO_3-N: ++					
24	*P. australis*	3 months	Textile bleaching WW	HFW	++ after 72 h	++ after 72 h	++	++	++	++	++ after 48 h	++ (Fe, Ni, Cd)	
29	*L. fusca*	1 year	Industrial WW (textile production)	HFW	++	++ after 48 h		++ after 48 h	++		++	++	++ for color·m^{-1}
34	*B. mutica*	8 days	Treated domestic WW mixed with raw industrial WW	FTW (site 1)	++	++		++	++ for PO_4-P			++ for Co, Fe, Mn + for Cd, Cr, Cu, Ni, Pb	++ for sulfates and chlorides; + for oil and grease
34	*B. mutica*	8 days	Treated domestic WW mixed with raw industrial WW	FTW (site 2)	++	++		++	++ for PO_4-P			++ for Cu, Pb + for Cd, Cr, Fe, Ni, Pb	+ for sulfates and chlorides; ++ for oil and grease
20	*P. australis*	15 days	DW spiked with phenol	FTW	++	++	++						++ for phenol
18	*P. australis*	20 days	Textile bleaching WW	FTW	++	++				++	++	+, but sign. Unclear	++ for color·m^{-1}
16	*B. mutica*	35 days	Domestic + industrial WW spiked with heavy metals	FTW	++	++						++ (Fe, Mn, Ni, Cr, Pb)	
16	*L. fusca*	35 days	Domestic + industrial WW spiked with heavy metals	FTW	++	++						++ (Fe, Mn, Ni, Cr, Pb)	
16	*P. australis*	35 days	Domestic + industrial WW spiked with heavy metals	FTW	++	++						++ for Fe, Mn, Pb; − for Ni, Cr	
16	*T. domingensis*	35 days	Domestic + industrial WW spiked with heavy metals	FTW	++	++						++ for Fe, Mn, Pb; − for Ni, Cr	
26	*B. mutica*	42 days	WW from crude oil production	FTW	++	++					++		++ for crude oil
26	*P. australis*	42 days	WW from crude oil production	FTW	++	++					+		++ for crude oil
26	*T. domingensis*	42 days	WW from crude oil production	FTW	++	++					+		++ for crude oil
26	*L. fusca*	42 days	WW from crude oil production	FTW	++	++					++		++ for crude oil
17	*P. australis*	90 days	Diesel conta-minated water	FTW	++	++	+			−			++ for phenol
25	*P. australis*	2 years[a]	Industrial WW (textile production)	FTW	+	+		++	+ for PO_4^{2-}	+	++	++	+ for chlorides, sulfates, phenol, color per meter

++: removal efficiency of inoculated system is significantly higher; +: removal efficiency of inoculated system is higher, but not significantly; −: removal efficiency of inoculated system is lower, but not significantly; −-: removal efficiency of inoculated system is significantly lower.

[a] Results obtained in the 2nd year of operation when the system was stable/at its optimum.

potentially transient and time-dependent nature of the microbial inoculation effects on TW efficiency, and the need to consider temporal dynamics in future bioaugmentation studies.

The method of bacterial inoculation also differed across studies, and could impact TW efficiency. Shao et al. (2016) compared liquid inoculation to bacterial inoculants immobilized on a microporous polyurethane support, and showed that microcosms with immobilized inoculants lowered COD, TP, TN and mineral N more efficiently. It is important to identify not only the most suitable bacterial inoculants to provide services in TWs, but also the most efficient and operationalizable methods of inoculation.

4.2.4. Effect of plant presence and identity

Among the studies on bacterial inoculation considered here, ten included unplanted controls, which make it possible to isolate the effect of plants on TW efficiency quite specifically. Overall, the effect of bacterial inoculation was highly contingent upon the presence of plants. For example, study Shahid et al. (2020) compared unplanted FTW microcosms to treatments inoculated with *Phragmites australis*, *Brachiaria mutica*, *Typha domingensis*, or *Leptochloa fusca*, for crude oil removal efficiency. The planted/inoculated microcosms showed a significantly better removal efficiency than the unplanted/inoculated ones, showing that bacterial inoculants alone may not necessarily improve FTW efficiency. However, for COD removal, the unplanted/inoculated controls performed as well as the planted/non-inoculated microcosms with *Brachiaria mutica* and *Leptochloa fuchsa*, showing that experimental additions of bacteria or plant-based stimulation of resident bacterial communities may have equivalent impacts in this context. Conversely, Fahid et al. (2020) showed superior efficiency for planted but uninoculated treatments as compared to unplanted but inoculated treatments, suggesting in this case that the presence of plants was more important than that of a bacterial inoculant. This shows how individual impacts of plants and bacterial inoculants, and their interactive effects, can differ from study to study, which may be tied to many factors, such as the nature of the contaminant, or the density and community structure of the bacterial inoculant and of the natural resident bacterial community. Regarding the impact of plant identity on bioaugmented TW efficiency, only two studies compared different plant species under the same conditions, which does not enable generalizable interspecific comparisons.

4.3. Combined effects of AM fungal and bacterial inoculants

To our surprise, only one study investigated both the effect of AM fungal and bacterial inoculation in parallel (Lingua et al., 2015). In that study, *Phragmites australis* in mesocosm scale tanks filled with 0.32 m^3 of cobbles (diameter 8–15 mm) were inoculated with *Funneliformis mosseae* or *Pseudomonas* under 4 levels of KNO_3 at a constant tap water level. Results showed that bacterial and AM fungal inoculation had roughly similar impacts on TW efficiency, as both improved performance over uninoculated controls. However, since the study did not include factorial combinations of AM fungi and bacteria, it is impossible to make any inferences about the potentially combined effect of AM fungi and bacteria. This clearly deserves further attention, as a vast body of literature exists on interactions between AM fungi and other colonists of the plant rhizosphere (e.g., Fitter and Garbeye, 1994; Requena et al., 1997; Artursson et al., 2006).

5. Synthesis and future research perspectives

Although results of research on bioaugmentation of TWs through mycorrhizal or bacterial inoculation are preliminary, they show some promise. It is surprising, however, that studies on AM fungal inoculation to date have barely targeted the most relevant parameter for treatment wetlands, i.e., treatment performance. AM fungal inoculation does seem to have a positive effect on *P. australis* biomass production, but more studies are required to further understand the context-dependent nature of mycorrhizal interactions in TWs. As many TWs are intended to treat waters with high nutrient loads and high COD and BOD (and thus with low oxygen availability), it is quite possible that this interaction essentially becomes a parasitism of the plant (e.g., Johnson et al., 1997; Chagnon and Brisson, 2017), which could limit plant biomass production and thus plant-derived benefits of TWs, thereby posing one of the challenges of bioaugmentation. On the other hand, AM fungi could improve plant performance in metal-polluted TWs, thus promoting plant-derived benefits. Future studies should disentangle the identity of resident AM fungi naturally colonizing TWs (e.g., Fester, 2013; Calheiros et al., 2019), and the traits that allow them to be successful in such unusual habitats with low-oxygen, flooded conditions (Chagnon and Brisson, 2017). Such studies might reveal that cosmopolitan AM fungi comonly used in inoculation trials (i.e., *Rhizophagus irregularis* and *Funneliformis mosseae*) are already quite abundant in uninoculated TWs, in which case there would be few benefits to artificially inoculating TWs with these taxa specifically.

AM fungi are not the only fungal endophytes colonizing wetland plants' roots. In both natural and constructed wetlands, non-mycorrhizal fungi belonging to a wide variety of fungal phyla are often observed (Weishampel and Bedford, 2006; You et al., 2015; Dolinar et al., 2016; Janowsky et al., 2019). Their ecological functions, however, remain elusive (Mandyam and Jumpponen, 2005). While there is solid evidence in phytoremediation that root endophytic fungi can promote plant performance by immobilizing contaminants (Zahoor et al., 2017), mobilizing inorganic nutrients (Li et al., 2012), altering plant hormonal balance (Deng and Cao, 2017) or degrading organic contaminants (Mandyam and Jumpponen, 2005), analogous evidence for treatment wetlands seems to be lacking. Yet, their high frequency of occurence, sometimes higher than that of AM fungi (Weishampel and Bedford, 2006), calls for a better appreciation of their role and potential as bioinoculants.

Regarding bacterial inoculation, the vast majority of indicators used to quantify TW efficiency showed upward trends upon inoculation. However, many unknowns remain regarding the long-term persistence of the inoculants, and the temporal trends in bacteria-derived benefits. Are these short-term and transient? How stable can assembled microbiomes be? Is it possible that the microbiome switches to another alternative stable state after several months, one that could be much less beneficial than the one established during the initial weeks post-inoculation? Data in this regard is scarce and should be the focus of future research, as TWs are intended to be durable solutions to a sustained environmental challenge, i.e., treating constant/periodic contaminated effluents. There is a need for longer-term assessments of microbial community structure and services provided by bioaugmented TWs.

A difficulty generally encountered when evaluating the effects bioaugmentation between the different studies is the blending of bioaugmentation with biostimulation. The latter refers to the use of various amendments (e.g., nutrients, organic matter) to improve the development of plants and/or microbes (Azubuike et al., 2016). As seen in the studies on mycorrhization, the different environmental contexts created through biostimulation (e.g., fertilization regimes) can alter plant response to AM fungi, and thus create an additional layer of complexity, which needs to be taken into account when trying to anticipate the potential benefits of inoculating TWs with AM fungi.

Another limitation in the literature is the lack of studies on large-scale systems (>5m^2, Brisson and Chazarenc, 2009). Eighty-six % of the studies on mycorrhization and 71% of those on bacterial inoculation were performed in microcosms (mostly in pots and buckets) with at most a few plants per unit. This increases edge and container effects, with a concurrent loss of ecological relevance (Fraser and Keddy, 1997). Plants in microcosms do not experience the effects of neighboring plants on light interception and growth allometry, and root dispersion can be affected by crowding on inner surfaces (Brisson and Chazarenc, 2009). For these reasons, while microcosms may be useful to determine broad patterns and proofs of concepts, for application

purposes, validation must be conducted in larger scale systems. We found no bioaugmentation study conducted in large, full-scale or pilot-scale TWs. For bacterial inoculation, at least a third of the experiments were conducted in mesocosms (medium size units), which allow better transfer of the results. The only relevant published large-scale study (Austin et al., 2019) demonstrated the applicability of bacterial inoculation to improve nitrification in cold temperatures.

Replicating the experimental units allows statistical testing and increases confidence that the differences detected in biomass development or pollutant removal are systematic and due to the treatment. For mycorrhization, more than half of the studies used well-replicated experiments (from 5 to 8 replicates), all performed in microcosms (Stevens and Peterson, 2007; Stevens et al., 2002; White and Charvat, 1999; Liang et al., 2018, 2019; Tang et al., 2001; Hu et al., 2020; Ipsilantis and Sylvia, 2007). This was not the case for the studies on bacterial inoculation, for which only three of 24 studies were replicated comparably (six times: Hussain et al. (2018a, 2018b, 2019)).

Specific plant species – inoculant combinations were tested a maximum of two times in the studies on mycorrhization (Table 1) and three times for bacterial inoculation (Table S2), but in most cases only once or twice, which limits generalizability of results. However, study duration also seems to have an impact on the results. While some studies began the inoculation procedure immediately, others waited for the system to achieve maturity. Several studies observed a change over time; e.g. Hussain et al. (2019) pointed out that removal performance of the tested HFCWs and VFCWs was optimal in the initial 3-month period, while it declined in subsequent months. At the same time, plant health status deteriorated, with plant shoots starting to turn yellowish. It is, therefore unclear how long a system treating industrial wastewater survives, and i) in the case of mycorrhization, how long artificial mycorrhization is more prevalent than natural mycorrhization and ii) how long bacterial inoculants can be measured in the systems.

Given the limited data on the effect of bioaugmentation on treatment wetlands as presented in this study, we recommend further studies on several aspects, listed in Table 6.

6. Conclusions

Bioaugmentation of treatment wetlands (TWs) shows promising results based on the 39 studies investigated in this review, both in the form of mycorrhizal incolation of TW plant roots and also as bacterial inoculation of TW systems. Mycorrhizal inoculation seems to have a positive effect on *Phragmites australis* biomass production, but further studies are needed to confirm this for other wetland plant species. The benefits of mycorrhization have not been tested to date under the operating conditions of TWs and with wastewater as inflow. Future research should address the efficiency of AM fungal inoculants using wastewaters as inflow, to determine whether AM fungi can (1) persist and (2) be effective in nutrient-rich and oxygen-deprived waters (Chagnon and Brisson, 2017).

Research on bacterial inoculation is already further advanced, as more studies conducted in micro- and mesocosms mimicking TWs have been published. In many cases, bacterial inoculation can help to improve plant growth and enhance treatment efficiency in domestic as well as industrial wastewater. Further research is needed on the half-life of these improvements, their degree of success on a full scale and overall cost benefit.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.145820.

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Table 6
Questions for further studies based on current research gaps.

Topic	Question
Mycorrhizal fungi	
Treatment performance	1. Do AM fungi improve the performance of treatment wetlands?
Type of water treated	2. Are AM fungi also efficient in real wastewaters, with higher loads of nutrients? 3. What is the capacity for different wetland plants to autoregulate their AM symbioses under low-benefit conditions?
Inoculum source	4. Are inoculum levels insufficient to allow adequate colonization in real TWs? Can spontaneous colonization of TW plants by AM fungi be sufficient to ensure the AM fungal provision of benefits to hosts? 5. How do TW plants respond to a broader set of AM fungal strains from different families?
Study duration	6. How do benefits derived from AM fungi vary through time? Are AM fungi more beneficial once they are well established? 7. In the long run, can TWs with spontaneous mycorhization be just as efficient as artificially inoculated ones? If so, how much time is required for spontaneously colonized TWs to close the gap?
Bacteria	
Inoculation	8. Can bacterial inocula persist over the long term or do they have to be reinoculated periodically to ensure proper TW functioning? 9. What competitive load do inoculated strains experience from native bacterial colonizers of TWs?
Spatial scale	10. Are bacterial inocula still beneficial in full-scale systems? 11. Can inoculation-derived benefits outweigh the inoculum production and application costs in full-scale TWs?

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