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Inhibitory effect of municipal sewage on symbiosis between mangrove plants and arbuscular mycorrhizal fungi

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ABSTRACT: The impact of municipal sewage discharge on the symbiosis between arbuscular mycorrhizal fungi (AMF) and mangrove plants in different sections along 2 constructed mangrove belts was evaluated. Each section was 33 m long and 3 m wide and planted with *Kandelia obovata* or *Aegiceras corniculatum*, the 2 most common mangrove plants in South China. A greenhouse experiment comparing the colonization intensity of AMF among different mangrove plant species under wastewater treatment was also conducted. Typical arbuscular mycorrhiza structures were observed in most of the root samples collected from the constructed belts. In both belts, the AMF colonization intensities were significantly lower in the roots of plants close to the inlet than in those further away from the influent, suggesting that the colonization intensity of AMF was inhibited by municipal sewage discharge, and that inhibition was least pronounced in the effluent where the concentration of nutrients was lowest. AMF colonization intensities in the roots of *A. corniculatum* were significantly higher than those in the roots of *K. obovata*, which could be attributed to the fact that *A. corniculatum* provided more oxygen to support the AMF than did *K. obovata*, indicating the strong effects of the host species on AMF colonization. In both constructed wetland belts and greenhouse experiments, the AMF vesicle and arbuscular structures (the main functional structure of arbuscular mycorrhiza) appeared to be more sensitive to wastewater discharge than the hyphal structure, implying that sewage discharge would reduce the potential beneficial effects of AMF in mangrove ecosystems. This study provides useful information on the responses of AMF to sewage; this knowledge is important for the conservation and restoration of mangrove forests that are located close to human activities.

KEY WORDS: *Aegiceras corniculatum* · Arbuscular mycorrhizal fungi · AMF · Constructed wetland · *Kandelia obovata* · Wastewater

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) are associated with over two-thirds of plant species in a symbiotic interaction known as arbuscular mycorrhiza (AM), which is the most ecologically and agriculturally important symbiosis in terrestrial ecosystems (Fitter et al. 2011). In exchange for photosynthates provided by plant symbionts, fungal partners improve plant access to phosphorus,

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nitrogen and other nutrients (Smith & Read 2008). The presence and composition of AMF species can profoundly affect the diversity and productivity of plant communities on land (van der Heijden et al. 2008). In addition to their widespread distribution and functional significance in terrestrial ecosystems, the presence of AMF in wetland habitats has also been reported (Wilde et al. 2009, Wang et al. 2011). Colonization of AMF in wetland plants also produces a wide range of benefits to their plant partners (Miller & Sharitz 2000, Wang et al. 2010), suggesting the importance of AMF in wetland ecosystems.

Mangrove forests are important wetlands in intertidal zones that are located in tropical and subtropical regions. They create unique ecological environments that host rich assemblages of species, and they also protect and stabilize coastlines, enrich coastal waters, yield commercial forest products and support coastal fisheries (Das & Vincent 2009). Despite their great ecological significance, global mangrove areas are declining rapidly, mainly due to inappropriate anthropogenic activities (Polidoro et al. 2010). In China, mangrove forests are mostly distributed in southern coastal regions (Lin 1999). During the past 50 yr, large areas of these mangrove forests have been cleared for coastal development, urban construction and aquacultures, and total mangrove area has been severely reduced, from approximately 40 000 ha in the 1950s to 15 000 ha in the 1990s (Lin 1999). In recent years, local and national governments have paid more attention to protection and restoration programs for mangrove forests in these regions. AMF could significantly improve the growth of mangrove plants through the enhanced absorption of nutrients and other elements (Wang et al. 2010). Several studies have shown that AMF are ubiquitous in mangrove habitats despite the saline and micro-aerobic conditions in the rhizosphere of mangrove plants (Sengupta & Chaudhuri 2002, Wang et al. 2011, D'Souza & Rodrigues 2013a,b).

Mangrove forests, due to their proximity to dense human populations and inadequate protection, often receive domestic sewage and other types of wastewater (Polidoro et al. 2010, Wang et al. 2013). Information on the impacts of municipal sewage discharge on mangrove plants (Herteman et al. 2011), ciliate communities (Chen et al. 2009), crab and mollusc assemblages (Cannicci et al. 2009) and soil enzymes (Yang et al. 2008) have previously been published; however, the impact of wastewater on the symbiosis between AMF and mangrove plants remains unexplored. In terrestrial ecosystems, the inhibitory effects of high levels of phosphorus (P) and/or nitrogen (N) on the symbiosis between AMF and plants have been widely studied (van Diepen et al. 2011, Lin et al. 2012). There is also solid evidence showing that excess organic matter can be harmful to this symbiotic relationship (Sáinz et al. 1998). However, an appropriate application of organic matter could promote AMF colonization in host species (Hodge & Fitter 2010). So far, the effect of excess nutrients or organic matter on the symbiosis between wetland plants and AMF remains poorly understood.

We evaluated the impact of municipal sewage discharge on the symbiosis between AMF and mangrove plants. The colonization intensity of AMF and the density of AMF spores in 6 sections, along 2 constructed mangrove belts planted with *Kandelia obovata* and *Aegiceras corniculatum*, were investigated. A greenhouse experiment was also conducted to compare the colonization intensity of AMF between 2 host mangrove species under wastewater treatment and control.

MATERIALS AND METHODS

Expt 1: Investigation of AMF in 2 constructed mangrove wetland belts

Constructed mangrove wetland belts

Two subsurface-flow, constructed, mangrove belts for treating municipal sewage were built as a field trial in the Futian Nature Reserve of Shenzhen in South China (22° 32′ N, 114° 03′ E). Each constructed mangrove belt was 33 m in length, 3 m in width and 0.5 m in height and filled with stones (bottom, 20 cm deep), gravel (20 cm deep) and a mixture of mangrove soil and sand (surface, 10 cm deep). No sterilization procedure was applied to the stone, gravel, sand and mangrove soil used for wetland construction. Two native mangrove species, *Kandelia obovata* Sheue Liu & Yong [previously known as *Kandelia candel* and renamed after Sheue et al. (2003)] and *Aegiceras corniculatum* L. Blanco, were selected as the test plants because of their great potential for natural wastewater treatment (Wong et al. 1997) and wide distribution in the mangrove habitats of China. One-year-old individuals of each of the 2 mangrove species were transplanted into each belt in May 2005, with a distance interval of 0.5 m between individual plants. Three months after transplanting, wastewater collected from the surrounding premises was discharged into the inlet of each belt after settling in a sedimentation pond for 1 h. The hydraulic loading for each belt was 5 m^3 d^{-1}, and the hydraulic retention time was 3 d. Basic chemical and physical parameters and the concentrations of the main pollutants in the influent and effluent were monitored bimonthly during the study period, from August 2005 to June 2008. Three replicates of water samples were collected at each sampling time. The pH value, concentrations of dissolved oxygen (DO), suspended substance (SS), chemical oxygen demand (COD), biological oxygen demand over 5 d (BOD_5), total nitrogen (TN), ammonia-nitrogen (NH_3-N), total phosphorus (TP) and soluble phosphate (SP) were determined following the standard methods for analysis of water and wastewater (APHA 1989).

Sample collection and analysis

In June 2008, the juvenile nutritive roots of 3 individual mangrove plants and the surface soil (0 to 10 cm layer) full of roots (called rhizosphere soil) were collected, once each, at 0, 5, 10, 15, 20, 25 and 30 m distances from the inlet. Fine root samples were cleared in 10% w/v potassium hydroxide (KOH) at 90°C for 40 min and then stained with trypan blue. The total colonization of AMF (TC%) calculated by summing the number of occurrences of vesicles and arbuscules or hyphae (including times when the structures occurred together as a single record), hyphal colonization (HC%), vesicle colonization (VC%) and arbuscular colonization (AC%) were assessed using the magnified intersection method (McGonigle et al. 1990). Then 200 intersects on 40 root segments were scored under a compound microscope (Carl Zeiss, Axiostar plus). AMF spores from the rhizosphere soil sample (20 g, air-dried) were obtained following the wet sieving and decanting method (An et al. 1990). Intact and healthy spores were counted to assess the density of the AMF spores. Air-dried samples were used in this study because of the distance between the field site and the laboratory. Care was taken when drying soil samples to reduce the loss in viability of spores.

Expt 2: Greenhouse experiment

Experimental design

It is difficult to find a large area near the municipal sewage discharge point that is also close to the foreshore region where mangroves naturally colonize. Because of space limitations, municipal sewage collected from surrounding premises was only discharged to the treatment belt, and tap water control was not applied in Expt 1. A supplementary, small-scale greenhouse experiment was therefore conducted to evaluate the effects of sewage discharge on AMF colonization, with comparison to the tap water control. A constructed wetland system made of 18 PVC pots, each with a dimension of 50 cm (length) × 35 cm (width) × 25 cm (depth), filled with gravel (bottom, 5 cm deep) and mangrove sand (surface, 20 cm deep), was built and kept in a greenhouse. In order to better simulate the natural conditions, no extra AMF inoculums were added to the planting substrates. Water was pumped though a rubber pipe set at the bottom of each pot, with the overflow directed in an upward direction. Similar to the field experiment, the gravel, sand and pipes were not sterilized. The 18 pots were divided into 6 groups, each in triplicate, and arranged in a randomized, complete block design with 2 factors, plant species (*Kandelia obovata*, *Aegiceras corniculatum* and without plant) and treatments (wastewater treatment and tap water control). Because of the limited availability of plant materials, 2-yr-old individuals of *K. obovata* or *A. corniculatum*, instead of 1-yr-old plants as used in Expt 1, were utilized. To enable a better comparison between the results obtained from field and greenhouse experiments, plant coverage in Expt 2 was similar to that in Expt 1, with 15 plants transplanted from the nursery to each pot in September 2007 for Expt 2. The wastewater used in the second experiment was the same as that in Expt 1. Tap water of the same volume was discharged into the tap water control pots. The hydraulic loading for each pot was 5 m^3 d^{-1}.

Harvest and measurements

At the end of the 9 mo experiment, 4 plants of *Kandelia obovata* or *Aegiceras corniculatum*, from each pot, were harvested and the roots were carefully separated from the soil. Root samples were used to determine the intensity of AMF colonization, and rhizosphere soil (5 to 15 cm) was used for the determination of the density of AMF spores, using the same methods described in Expt 1.

Statistical analysis

A parametric 1-way ANOVA, followed by a least significant difference (LSD) test at a 5% confidence level, was used to determine any differences in AMF colonization intensity and spore density collected at the different distances from the inlet of each mangrove belt. The same test was used to compare the colonization intensity and spore density measurements between wastewater treatment and the tap water control in the greenhouse experiment. Two-way ANOVA was applied to analyse the effects of plant species and wastewater treatment on the AMF colonization intensity and spore density, with distance from the inlet as the covariate in Expt 1. The effects of plant species, wastewater treatment and their interactions in Expt 2 were also analysed using 2-way ANOVA. The differences between influent and effluent properties for each host plant were compared by a paired *t*-test at a Bonferonni-corrected significance level. All statistical analyses were performed using the commercial software SPSS 16.0.

Table 1. Basic physical and chemical parameters and the concentrations of major pollutants in the influent and effluent of 2 constructed mangrove wetland belts planted with *Aegiceras corniculatum* and *Kandelia obovata*. Mean and range (in parentheses) values of 3 replicates are shown; an asterisk indicates significant differences between influent and effluent for each host plant according to paired-samples *t*-tests with a Bonferonni-corrected significance level at $p \leq 0.05$. DO: dissolved oxygen; SS: suspended substance; COD: chemical oxygen demand; BOD_5: 5 d biological oxygen demand; TN: total nitrogen; NH_3-N: ammonia nitrogen; TP: total phosphate; SP: soluble phosphate

Parameters	Influent	Effluent	
		A. corniculatum	*K. obovata*
pH	7.55 (7.49 – 7.67)	7.38 (7.02 – 7.78)	7.45 (6.99 – 7.95)
DO (mg l^{-1})	1.82 (0.28 – 4.51)	4.58* (2.83 – 5.76)	4.05* (2.48 – 5.14)
Salinity (‰)	1.63 (0.34 – 7.34)	1.62 (0.34 – 4.80)	1.33 (0.31 – 2.90)
Conductivity (mS cm^{-1})	3.07 (0.70 – 13.60)	3.00 (0.48 – 4.26)	2.52 (0.62 – 5.42)
Redox potential (mV)	–155 (–453 – 68)	23.3* (–0.6 – 31.2)	15.1* (11.8 – 24.5)
SS (mg l^{-1})	48.1 (12.5 – 109.2)	5.3* (3.7 – 6.1)	4.8* (3.7 – 6.6)
COD (mg l^{-1})	133.6 (26.7 – 183.2)	37.8* (4.0 – 91.0)	42.0* (4.0 – 81.0)
BOD_5 (mg l^{-1})	62.1 (18.3 – 95.5)	13.6* (1.3 – 40.0)	13.8* (2.5 – 34.0)
TN (g kg^{-1})	15.58 (7.57 – 28.94)	7.98* (0.82 – 18.98)	8.25* (1.43 – 15.70)
NH_3-N (mg kg^{-1})	11.46 (7.12 – 26.24)	6.00* (0.61 – 13.24)	7.27* (2.62 – 15.11)
TP (g kg^{-1})	1.28 (0.94 – 2.31)	0.45* (0.13 – 1.04)	0.64* (0.27 – 2.08)
SP (mg kg^{-1})	1.06 (0.66 – 1.94)	0.32* (0 – 0.88)	0.48* (0.15 – 1.86)

RESULTS

AMF colonization intensity and spore density in the constructed mangrove belt (Expt 1)

In both mangrove belts, the concentrations of COD, BOD_5 and nutrients (TN, NH_3-N, TP and SP) in the effluent were significantly lower than that in the influent (Table 1), indicating that wetland belts gradually purified municipal sewage as it passed through. The removal of organic matter and nutrients (N and P) amounted to between 50 and 70% for both constructed wetlands. Typical AM structures (hyphae, vesicle and arbuscules) were observed in most of the root samples of *Kandelia obovata* and *Aeqiceras corniculatum*, collected at different distances along the belt (Figs. 1 & 2). No typical structures of dark septate endophytes were found. In the *A. corniculatum* belt, the total colonization of AMF, hyphal colonization, vesicle colonization and arbuscular colonization were significantly lower in the roots of the plants closer to the inlet than in those further away from the influent (Fig. 1). Similar trends were observed in the constructed *K. obovata* wetland belt (Fig. 2), although the AMF colonization intensities in the roots of *K. obovata* (TC%: 2.5–22.5%, HC%: 2.5–15.5%, VC%: 0–7.4% and AC%: 0–2.8%) were generally lower than those in the roots of *A. corniculatum* (TC%: 15.5–63.5%, HC%: 13.5–48.0%, VC%: 3.5–27.5% and AC%: 1.0–18.0%). In both belts, the AMF hypha was the dominant structure in the roots of plants close to the inlet, while the vesicle and arbuscular structures were either rarely observed or absent. This implies that the AMF vesicle and arbuscular structures were more sensitive to wastewater than the hyphal structure. Results from 2-way ANOVA showed that the effects of plant species and wastewater on the AMF colonization intensities were statistically significant ($p < 0.01$; Table 2).

In both constructed wetland belts, AMF spores were recorded in most of the soil samples, but the spore densities were comparatively low, <10 spores per 20 g soil (Fig. 3). AMF spores distributed randomly, with no significant difference in spore densities among the soil samples collected across the inlet ($p > 0.05$). Significant differences in spore densities did not occur between the 2 wetland belts either ($p > 0.05$). Two-way ANOVA also showed that neither plant species nor wastewater treatments had any significant effects on the density of AMF spores ($p > 0.05$; Table 2).

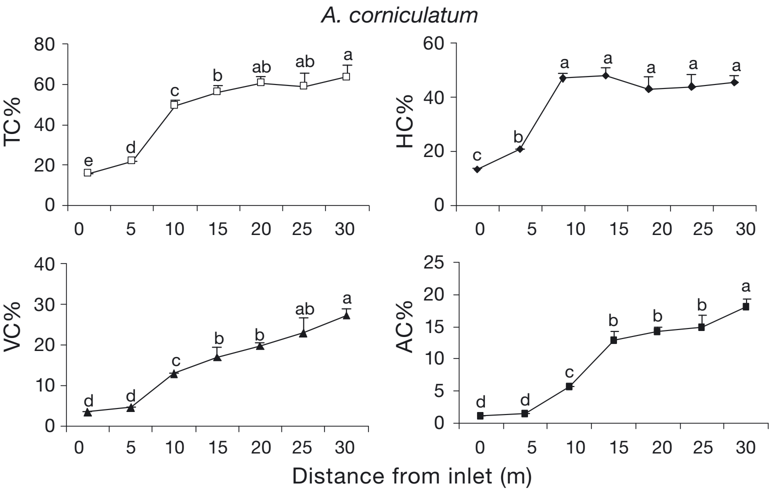

Fig. 1. Colonization intensity of arbuscular mycorrhizal fungi (AMF) in the roots of *Aegiceras corniculatum* at different distances from the sewage inlet in the constructed mangrove belt. Different letters in each panel indicate the colonization intensities of AMF in the roots of the host species are significantly different at the 0.05 probability level according to 1-way ANOVA. TC%: percent total colonization rate (calculated by summing the number of occurrences of vesicles, arbuscules, or hyphae, including times when the structures occurred together as a single record); HC%: percent colonization rate of hyphae; VC%: percent colonization rate of vesicles; AC%: percent colonization rate of arbuscules

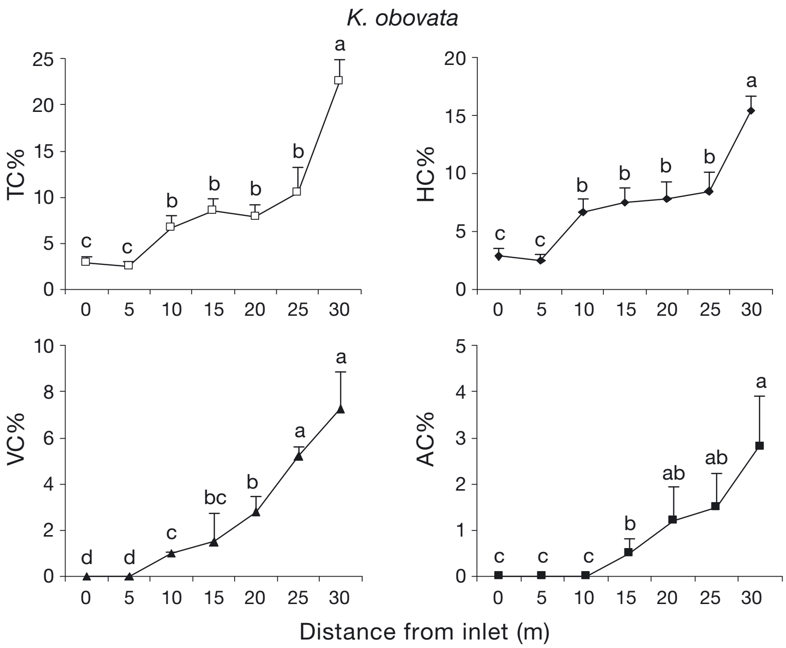

Fig. 2. As above, but for *Kandelia obovata*

AMF colonization intensity and spore density in the greenhouse experiment (Expt 2)

The AMF colonization intensities (hyphal, vesicle, arbuscular and total colonization percentages) in mangrove roots, irrespective as to whether it was *Aegiceras corniculatum* or *Kandelia obovata*, in wastewater treatment, were significantly lower than those in the tap water control ($p < 0.01$) (Fig. 4), indicating that wastewater discharge inhibited the symbiosis between AMF and mangrove species. TC% and VC% in *A. corniculatum* roots were significantly higher than those in *K. obovata* roots, both in the control and the wastewater treatments. When comparing the results between Expts 1 and 2, the AMF colonization intensities for both *A. corniculatum* and *K. obovata* were at similar levels (Figs. 1, 2 & 4). Results from 2-way ANOVA showed that the effects of plant species and wastewater on AMF colonization intensities were statistically significant (Table 2), except for the effect of wastewater on HC% ($p > 0.05$). However, 2-way ANOVA showed that neither species nor wastewater treatment had any significant effects on the density of AMF spores (Table 2), and the AMF spore densities in the samples collected from all pots were relatively low (most were <10 spores per 20 g soil; Fig. 5).

DISCUSSION

Many environmental factors have the potential to affect the colonization of AMF in host species (Smith & Read 2008). In terrestrial ecosystems, high P supplies suppress the symbiosis between AMF and plants (Smith et al. 2011), which could be interpreted as an active strategy of the plant to limit carbohydrate consumption of the fungus by inhibiting its proliferation in roots in which a set of symbiosis-related genes are involved (Breuillin et al. 2010). High N levels also inhibit AMF colonization, as the relative allocation of carbohydrates to AM structures could be reduced by N enrichment (Johnson et al. 2003), and high concentrations of NH_4^+ could be toxic to AMF colonizing in roots, inhibiting the germination of AMF spores (Cornejo et al. 2007). In wetland ecosystems, AMF colonization could also be affected by a variety of environmental factors, such as hydrological conditions (Miller & Bever 1999, Wang et al. 2011), nutrients (Wang et al. 2010), pH and electric-conductivity levels (D'Souza &

Table 2. Univariate 2-way ANOVA analyses showing the effects of mangrove species and wastewater treatment on arbuscular mycorrhizal fungi (AMF) colonization intensity and density of AMF spores. Distance was used as a covariate in comparing the effects of host species and wastewater treatment in Expt 1. See Fig. 1 for abbreviations. DS: density of AMF spores (no. per 20 g soil); *p < 0.05; **p < 0.01, NS: not significant

Variables	TC%		HC%		VC%		AC%		DS	
	F	df	*F*	df	*F*	df	*F*	df	*F*	df
Expt 1										
Species	90.47**	1	78.33**	1	78.36**	1	42.15**	1	3.21	1
Wastewater	60.99**	6	31.67**	6	52.92**	6	27.71**	6	2.23	6
Expt 2										
Species	26.79**	1	22.92**	1	16.94**	1	29.5**4	1	0.38^{NS}	2
Wastewater	15.29**	1	0.47^{NS}	1	41.20**	1	20.51**	1	0.34^{NS}	1
Interaction	3.62^{NS}	1	0.08^{NS}	1	10.96*	1	18.51**	1	0.88^{NS}	2

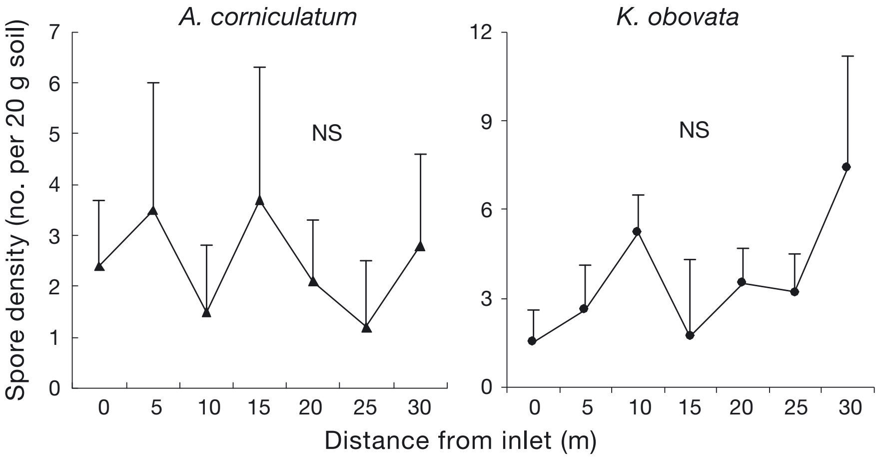

Fig. 3. AMF spore density in the rhizospheric soil at different distances from the sewage inlet in 2 constructed wetland belts planted with *Aegiceras corniculatum* or *Kandelia obovata*. NS: not significant

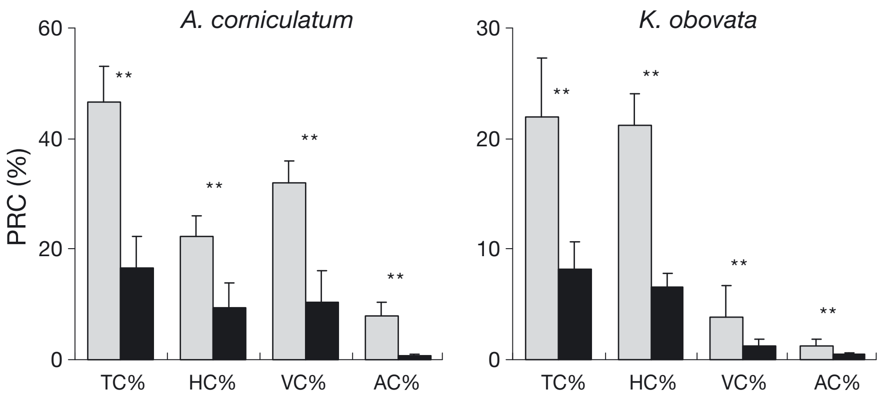

Fig. 4. Percentage root colonization (PRC) of AMF in the roots of *Aegiceras corniculatum* and *Kandelia obovata* under wastewater treatment (light grey bars) and tap water control (dark grey bars). See Fig. 1 for abbreviations. **p < 0.01

Rodrigues 2013b). The present study revealed that municipal sewage discharge significantly inhibited the colonization intensity (including hyphal, vesicle and arbuscular colonization) of AMF in 2 mangrove species, and the inhibition was least at the effluent, where the concentration of nutrients was lowest. Comparatively high levels of N, P and organic matter in the sewage should partially account for this inhibitory effect, considering that the inhibitory effects of high nutrients on AMF have been widely reported in terrestrial ecosystems (Hodge et al. 2010, Fitter et al. 2011). Further, in wetland ecosystems, the survival of AMF relies on the oxygen provided by the root of wetland plants under flood conditions (Miller & Bever 1999, Wang et al. 2010). Brzezinska et al. (2006) found that the discharge of wastewater rich in nutrients and organic matter stimulates microbial activities and consumption, which leads to rapid depletion of oxygen in soil. Similarly, wastewater may decrease the radial oxygen loss of the roots of mangrove species and reduce the amount of oxygen released to either the rhizosphere or the root's surroundings (Pi et al. 2010a,b). The decrease in oxygen content in the rhizosphere of mangrove plants might explain the inhibitory effects of sewage on the colonization of AMF.

The present study showed that AMF vesicles and arbuscules were more sensitive to wastewater discharge than were hyphal structures. Smith & Read (2008) reported that the formation of AMF vesicles and arbuscules in roots is achieved by differentiation, such as twisting, branching and expansion of AMF hyphae in the inner cortical cells. The higher sensitivities of vesicles and arbuscules implies that sewage discharge might inhibit their formation processes of hyphal structures; however, the details and the mechanisms involved remain unknown. So

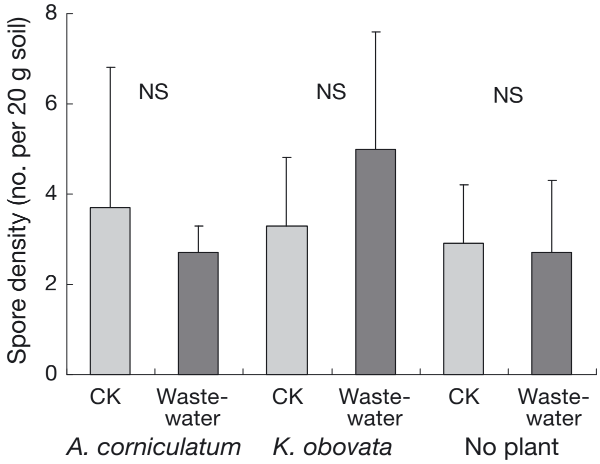

Fig. 5. Spore density of AMF in the soil of the wastewater treatment and tap water control (CK) in the 3 constructed wetland belts with *Aegiceras corniculatum*, *Kandelia obovata*, or no plants. NS: not significant

far, only the study by Pawlowska & Charvat (2004) reported the effects of heavy metal stress on different stages of AMF life history within roots. The role of AMF in mangrove ecosystems is still largely unknown, though Wang et al. (2010) found that AMF can improve the nutrient absorption of some mangrove plants. Therefore, it is difficult to evaluate, comprehensively, the ecological consequences of AMF inhibition (or extinction) in mangrove ecosystems caused by sewage discharge. Nevertheless, it is reasonable to believe that the inhibitory effects of sewage discharge on AMF colonization, especially on the arbuscular and vesicle structures, are related to the nutrient exchanges between AMF and their host plants and the nutrient storage for AMF, respectively (Smith & Smith 2011). This inhibition would greatly reduce the potentially beneficial effects of AMF on mangrove ecosystems. The effects of sewage discharge on the growth of mangrove plants have been evaluated in several studies (Wong et al. 1995, Herteman et al. 2011), but no congruent view has been reached. The results of the present study provide knowledge important for managing the conservation and restoration of mangrove forests, especially those subject to human disturbance.

Strong effects of the host species on AMF colonization were found in the present study, and AMF colonization intensities in *Aegiceras corniculatum* were significantly higher than in *Kandelia obovata*. The effects of host species on AM symbiosis could be attributed to many factors, including the differences in plant physiology, root morphological structure and the components of root exudates (Smith & Read 2008). Wang et al. (2010) found little or no AMF colonization on the young seedlings of some mangrove species, where the aerenchyma was not yet well developed. Nutrient conditions and planting space of host plants could also influence AM symbiosis, since this could affect the allocation of C sources from plants to the fungal symbiont (Smith & Read 2008). The higher AMF colonization intensity in *A. corniculatum* roots could be partly due to the release of more oxygen, indicated by the higher concentration of dissolved oxygen and lower negative redox potential in the constructed wetland belt planted with *A. corniculatum* wetland (Table 1). The mechanism involved and the importance of oxygen in AMF merits further research. Additionally, *A. corniculatum*, when compared to *K. obovata*, had higher efficiency in removing N and P from sewage (Table 1). This may also contribute to the higher AMF colonization intensity within the roots of *A. corniculatum*, since high nutrient levels could inhibit AMF colonization (Fitter et al. 2011). Further studies are still needed to elucidate the reasons behind this.

In the present study, the density of AMF spores was comparatively low and spores were distributed randomly in the constructed wetland belt. In recent field investigations of the mangrove ecosystems in Shenzhen and Zhuhai, China (Y. Wang et al. unpubl.) similar results were obtained. The environmental factors affecting the distribution of AMF spores have been widely reported, but mostly in terrestrial ecosystems (Oehl et al. 2009). Only a few researchers have investigated the distribution pattern of AMF spores in wetland ecosystems (Miller & Bever 1999, Wilde et al. 2009). D'Souza & Rodrigues (2013b) found that the spore density and species richness of AMF in a mangrove system in Goa, India, was co-affected by season and host plants. The hydrological conditions, such as soil moisture and water depth, were also very important in determining the distribution patterns of AMF spores in wetland ecosystems (Miller & Bever 1999, Wilde et al. 2009). In the present study, no significant change over distances from the influent to the effluent, nor between wastewater treatment and control was determined, which might be partially due to the low AMF spore density throughout the study. Further, considering the wide application of sand and gravel in the construction of the wetlands, the low AMF spore density and their random distribution pattern might be related to the frequent water irrigation. More in-depth studies on why the present mangrove habitats had such low AMF spore counts and what role AMF colonization plays in mangrove ecosystems, particularly those with high levels of sewage pollution, should be carried out.

In recent decades, constructed wetlands have been increasingly used for wastewater treatment due to their low maintenance requirements and construction costs (Kivaisi 2001). As the major component of a constructed wetland, wetland plants play an important role in removal of contaminants (Vymazal 2011). In the present study, constructed wetlands planted with either *Kandelia obovata* or *Aegiceras corniculatum* both showed high efficiency in the removal of organic matter and nutrients (Table 1). These results agree with previous findings that mangrove plants, due to their fast growth rate, high demand for nutrients and rich oxygen supply from their extensive roots and aerial root system, are capable of removing wastewater-borne pollutants efficiently (Wong et al. 1997, Yang et al. 2008, Zhang et al. 2010). Constructed mangrove wetland has a high application potential in wastewater treatment.

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