Diverse and abundant arbuscular mycorrhizal fungi in ecological floating beds used to treat eutrophic water

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Abstract
An increasing number of investigations have shown the universal existence of arbuscular mycorrhizal fungi (AMF) in aquatic ecosystems. However, little is known about the accurate distribution and function of AMF inhabiting aquatic ecosystems, especially ecological floating beds (EFBs), which are constructed for the remediation of polluted water bodies. In this study, we collected root samples of Canna generalis, Cyperus alternifolius, and Eichhornia crassipes from three EFBs on two eutrophic lakes in Wuhan, China. We aimed to investigate the resources and distribution of AMF in EFBs using Illumina Mi-seq technology. A total of 229 operational taxonomic units (OTUs) and 21 taxa from 348,799 Glomeromycota sequences were detected. Glomus and Acaulospora were the most dominant and second most dominant genera of AMF in the three EFBs, respectively. Different aquatic plant species showed varying degrees of AMF colonization (3.83–71%), diversity (6–103 OTUs, 3–15 virtual taxa), and abundance (14–57,551 sequences). Low AMF abundance, but relatively high AMF diversity, was found in C. alternifolius, which is usually considered non-mycorrhizal. This finding indicated the high accuracy of Illumina sequencing. Our results also revealed a lognormal species abundance distribution that was observed across AMF taxa in the three plant species. The AMF community composition was closely related to nitrogen and phosphorus contents. Overall, our data show that EFBs harbor diverse and abundant AMF communities. Additionally, the AMF community composition is closely related to the water quality of eutrophic lakes treated by the EFBs, indicating the potential application of AMF in plant-based bioremediation of wastewater.

Keypoints

• Aquatic plants in EFBs harbor diverse (229 OTUs) and abundant (348,799 sequences) AMF.
• Different plant species host different taxa of AMF. Cyperaceae, originally considered non-mycorrhizal, may in fact be a variable mycorrhizal plant family.
• The AMF community composition in EFBs is closely related to nutrient concentrations (nitrogen and phosphorus).

Keywords Glomeromycota · Arbuscular mycorrhizal fungi · Aquatic ecosystem · Ecological floating beds · Illumina sequencing · Community composition

Introduction
Arbuscular mycorrhizal fungi (AMF) are an ancient group of root symbionts, the origin of which synchronized with the occurrence of terrestrial plants (Bonfante and Genre 2008). Arbuscular mycorrhizal (AM) symbiosis involves approximately 80% of terrestrial plants and approximately 250 morphologically defined or 350 to 1000 molecularly defined AMF (Kivlin et al. 2011; Öpik et al. 2014). AMF benefit the establishment of plants by improving nutrient acquisition of plants and soil quality, promoting the adaptability of plants under various stressful conditions and enhancing the stability of host community composition (Artursson et al. 2005).
Although AMF have been extensively studied in terrestrial ecosystems, they have been poorly documented in aquatic ecosystems. Previous studies have suggested that AMF have little importance in aquatic ecosystems (Cooke et al. 1993; Khan 1993; Peat and Fitter 1993; Reid and Bowen 1979). This conclusion may be based on the restricted oxygen in saturated aquatic soils, which is detrimental to the survival of AMF (Crawford 1992). Additionally, submerged and floating aquatic plant species can obtain inorganic nutrients from the water and substrates, and through shoot and root surfaces (Sculthorpe 1967). Therefore, aquatic plants may not require AMF to help them obtain nutrients. Over the last two decades, an increasing number of investigations have shown the presence of AMF in aquatic ecosystems. AM symbiosis has been found in 99 families of wetland plants and even in submerged aquatic plants from various aquatic and wetland habitats (Xu et al. 2016). Several wetland plant species (e.g., those belonging to the families Cyperaceae, Chenopodiaceae, and Plumbaginaceae), which are generally considered non-mycorrhizal plants (Hirsch and Kapulnik 1998), have shown high levels of AMF colonization in their roots (Hildebrandt et al. 2001; Muthukumar et al. 2004). Therefore, the distribution and function of AMF in aquatic ecosystems need to be further investigated.

Most studies on the diversity of AMF in aquatic ecosystems were performed using morphological methods (D’Souza and Rodrigues 2013; Weishampel and Bedford 2006). Only a few studies were conducted by molecular approaches, such as restriction fragment length polymorphism (Wolfe et al. 2007), temporal temperature gradient gel electrophoresis (Likar et al. 2009), denaturing gradient gel electrophoresis (Calheiros et al. 2019), clone libraries (Guo and Gong 2014), and single-stranded conformation polymorphism (Nielsen et al. 2004). However, the biggest limitation of these methods is that they focus only on the dominant AMF taxa in complex microbial communities and are inadequate to detect a relatively low abundance of AMF taxa (Douterelo et al. 2014; Pimentel et al. 2017; Zinger et al. 2012). The lack of resolution of these methods may lead to neglecting rare species and to one-sided conclusions. In recent years, next-generation sequencing (NGS) technologies have been applied in some research on AMF communities owing to their comprehensiveness and accuracy in analyzing soil microbial community composition. However, only a few studies have focused on AMF in wetland and lakebed habitats (Moora et al. 2016; Silvani et al. 2017).

The ecological floating bed (EFB) is a promising ecological engineering tool for remediating eutrophic (Liu et al. 2020; Lyu et al. 2020; Wu et al. 2016), organically polluted (Chen et al. 2012) and heavy metal–polluted (Richter et al. 2016) water bodies. EFBs have many advantages, such as occupying a small amount of land, being minimally affected by fluctuating water levels, convenient management, being economical and efficient, and having a good landscape effect (Chen et al. 2016). The ecological functions of EFBs have been studied in situ, in mesocosms and in the laboratory. To the best of our knowledge, there has been only one study (Stenlund and Charvat 1994) that investigated AMF diversity in EFBs using a morphological method and reported low levels of AMF in EFBs.

In the present study, root samples of aquatic plants were collected from three EFBs on two eutrophic lakes located in Hubei province of China. This study aimed to investigate the AMF community composition in EFBs that treat eutrophic lake water, using the Illumina Mi-seq technique. Specifically, we aimed to examine the resources and distribution of AMF in EFBs that were planted with mycorrhizal plant species (Canna generalis and Eichhornia crassipes). We also aimed to determine if AMF was present in the roots of Cyperus alternifolius, which was considered non-mycorrhizal by morphological identification. We hypothesized that the roots of aquatic plants inhabiting EFBs have a high diversity and abundance of AMF, and that different aquatic plant species show varying degrees of AMF colonization, diversity, and abundance.

Materials and methods

Study area and sampling

Donghu Lake and Nanhu Lake are located in northeast Wuhan, which is the largest city in central China. Donghu Lake is the largest urban lake in Wuhan, covering an area of 33.7 km², with an average depth of 2.8 m and a maximum depth of 4.8 m. Nanhu Lake, which is the third largest urban lake in Wuhan, covers an area of 7.6 km² and has a maximum depth of 3.2 m. In the 1990s, with the rapid development of industrialization and urbanization in Wuhan, anthropogenic activities became increasingly frequent. Large quantities of industrial wastewater, agricultural effluents, sewage, and domestic wastewater were directly discharged into Donghu Lake and Nanhu Lake without treatment. Discharge of excessive inorganic nutrients (i.e., nitrogen and phosphorus) led to eutrophication, algal blooms, and habitat degradation in the two lakes. To restore the ecosystem structure and reduce the eutrophication of the Donghu and Nanhu lakes, the local government constructed some EFBs on the two lakes. The constructed EFBs were composed of hundreds of small ecological floating plates (0.5×0.5 m) in which aquatic plants from different nursery companies were planted (plants in Donghu Lake were from Chunyan Agricultural Science and Technology Co., Ltd., Foshan, China; plants in Nanhu Lake were from Bashu Aquatic Flower Base, Chengdu, China) (Supplemental Fig. S1). Three types
of plant species (C. generalis, C. alternifolius, and E. crassipes) were found on these EFBs, and the solid substrates of the EFBs were mostly silt and ceramic. Although the EFBs had been in operation for many years, the aquatic plants in them were still growing well.

The first EFB (30 × 30 m) in Donghu Lake (30°34′N, 114°22′E) was divided into two parts depending on the species of aquatic plants. One part was planted with C. generalis (EA), and the other part was planted with C. alternifolius (EB). Root samples were collected from three sites (EA1, EA2, and EA3) in EA and three sites (EB1, EB2, and EB3) in EB, and each sampled site was at least 5 m apart from the other. No sampling was conducted at the boundary between the two parts. The second EFB (20 × 20 m) was planted with C. generalis (SA), and the third EFB (20 × 20 m) was planted with E. crassipes (SB) in Nanhu Lake (30°30′12″N, 114°20′39″E). Root samples were collected from three sites (SA1, SA2, and SA3) in SA and three sites (SB1, SB2, and SB3) in SB, and each sampled site was at least 5 m apart from the other. Sampling was conducted in February 2019. Briefly, at each site (12 sites in total), five individual plants were randomly selected and dug out from a regularly spaced grid (4 × 4 m), and the roots from these five plants were pooled and used as a composite root sample for that site. Thus, a total of 60 individual plants were collected from the three EFBs. Each sample was collected with disposable equipment to avoid cross-contamination. Care was taken to collect only fine young roots because this is where most mycorrhizal colonization occurs (Smith and Read 2008). All of the samples were stored on ice and then transported to the laboratory. Roots of each sample were washed and rinsed with sterile water, dried on paper towels, and then divided into two parts. One part of the root samples was fixed in formalin-acetic acid-alcohol solution (formalin 5 ml + glacial acetic acid 5 ml + 70% alcohol 90 ml) to detect mycorrhizal colonization, and the other part of the roots was stored at −80 °C for subsequent DNA extraction. Water samples near the roots were also collected to evaluate the water quality at the same time.

**Determination of water quality**

Parameters including temperature, dissolved oxygen, pH, electrical conductivity, specific conductance, atmospheric pressure, total dissolved solids, salinity, and redox potential were measured in situ with a YSI 6600 V2 multiparametric sonde (Yellow Springs Instrument Co., Yellow Springs, OH, USA). A laboratory analysis of chemical oxygen demand (COD), ammonia nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N), nitrite nitrogen (NO₂⁻-N), total nitrogen (TN), inorganic phosphorus (IP), and total phosphorus (TP) was performed according to standard methods (EPAC 2002).

**AMF colonization analysis**

After the roots were rinsed with sterile water, they were stored in formalin-acetic acid-alcohol solution, cut into 1-cm fragments, and then stained according to the modified method of Phillips and Hayman (1970). Overall, 30 root fragments of 1 cm/sample were used to measure mycorrhizal colonization under a light microscope (Olympus Bx51, Tokyo, Japan) at 200× magnification. The frequency of mycorrhiza (F%), intensity of mycorrhizal colonization (M%), and arbuscule abundance in the root system (A%) were calculated according to the method described by Trouvelot et al. (1986). The M% was based on a five-class system ranking as follows: rare (n₁; < 1%), low (n₂; 1–10%), medium (n₃; 11–50%), high (n₄; 51–90%), and abundant (n₅; 91–100%). The A% was based on a three-class system ranking as follows: low (A₁; < 10%), medium (A₂; 11–50%), and high (A₃; 51–100%).

The F% was calculated as follows:

\[ F\% = \left( \frac{n_b}{30} \right) \times 100 \]

where \( n_b \) is the number of fragments with colonization by AMF.

The M% was calculated as follows:

\[ M\% = \left[ \left( \frac{0.95n_5 + 0.7n_4 + 0.3n_3 + 0.05n_2 + 0.01n_1}{30} \right) \right] \times 100 \]

where \( n_1, n_2, n_3, n_4, \) and \( n_5 \) are the number of root fragments at each level of colonization intensity.

Further calculations were as follows:

\[ m\% = M \times 30/n_b \]

\[ a\% = mA_3 + 0.5mA_2 + 0.1mA_1 \]

where \( A_1, A_2, \) and \( A_3 \) are the number of root fragments at each level of arbuscule abundance; \( mA_3, mA_2, \) and \( mA_1 \) are the % of \( m, \) rated \( A_3, A_2, \) and \( A_1, \) respectively, with \( mA_3 = [(0.95n_5A_3 + 0.7n_4A_3 + 0.3n_3A_3 + 0.05n_2A_3 + 0.01n_1A_3)/n_b] \times m, \) and the same was applied for \( A_2 \) and \( A_1. \)

\[ A\% = (a \times M)/100 \]

**DNA extraction and polymerase chain reaction amplification**

The methods used to extract AMF DNA and amplify AMF 18S ribosomal RNA genes were the same as those used in our previous studies (Ban et al. 2017; Xu et al. 2018). The primers used in the nested polymerase chain reaction (PCR) reaction were AML1 (5′-ATCAACTTCTGATGGAAGTTAGA-3′)/AML2 (5′-GAACCCAAACACTTTGGTTCC-3′)
(Lee et al. 2008) and AMV4.5NF (5′-barcode-AAGCTC GTAGTGAATTTCG-3′)/AMDGR (5′-CCCAACTATCCC TATTAATCAT-3′) (Cui et al. 2016; Lumini et al. 2010). The amplicons obtained from the nested PCR were 800 and 300 bp, respectively. All PCR amplifications were performed in triplicate (refer to Method S1 in the supplemental materials for details of PCR parameters).

**Illumina sequencing**

The methods of extraction, purification, and quantification of PCR amplicons were the same as those used in our previous study (Ban et al. 2017). Purified amplicons were pooled in equimolar amounts and paired-end sequenced (2 × 250) on an Illumina MiSeq platform (Personalbio, Shanghai, China) according to standard protocols (Caporaso et al. 2012). The raw reads were deposited into the NCBI Sequence Read Archive database (Accession Number: PRJNA550311).

**Processing of sequencing data**

QIIME (version 1.9.1) was used to demultiplex and quality filter the raw data (FASTQ files) (Caporaso et al. 2010) with some criteria (refer to Method S2 in the supplemental materials for the detailed criteria). The methods used to cluster the operational taxonomic units (OTUs), identify and remove chimeric sequences, identify the representative OTUs, evaluate the richness index, compute the overdominance (O) and inequitability (I), determine the form of distribution of taxonomic abundance, and conduct analysis for the relationships among species richness (S), Shannon entropy (H′), and the equitability index (E_{BG}) (i.e., SHE) were the same as those used in our previous study (Xu et al. 2018). We calculated the value (Pi − Pe)/Pe to quantify the highlights of taxonomic dominance in communities (Moebius-Clune et al. 2013), where Pi denotes the relative abundance of OTU i, and Pe was calculated as the ratio of 1 to the total OTU number (i.e., all of the OTUs in a perfectly even community have the same relative abundance). The value (Pi − Pe)/Pe of the top-ranked OTU was denoted as “overdominance” O, and the proportion of OTU with a negative value (Pi − Pe)/Pe was denoted as “inequitability” I. SHE analysis of the whole AMF assemblage was also carried out using S and H′ generated from rarefaction estimates by EstimateS, and the E_{BG} calculated from S and H′. The sequence reads were assigned to virtual taxa (VTs) by conducting a BLAST search against the MaarjAM and NCBI databases on January 2020 with the following criteria required for a match: sequence coverage ≥ 90%, sequence similarity ≥ 97%, and a BLAST e-value < 1e-50 (Moora et al. 2016; Öpik et al. 2009).

**Statistical analysis**

The COD and nitrogen and phosphorus concentration data were analyzed using IBM SPSS 25.0 (IBM Corp., Armonk, NY, USA). Significant differences between the four groups were detected using a one-way analysis of variance (ANOVA) (P < 0.05). Significant differences between means were determined by Duncan’s test (P < 0.05).

Species accumulation curves, Venn graphs, line graphs, histograms, and pie graphs were created using R software (version 3.6.1; Rstudio, Boston, USA). Non-metric multidimensional scaling (using the vegan package in R) and permutational ANOVA (using the adonis function in vegan) were used to partition variance in the 12 AMF communities. UniFrac dissimilarity (Lozupone and Knight 2005) was calculated using QIIME (Caporaso et al. 2010) and used as a measure of distance between pairs of AMF communities. Metastats analysis was conducted using Mothur software (version 1.30.1, the University of Michigan, Ann Arbor, USA) to perform pairwise comparison tests for the difference in sequence number between the four groups at the genus level (White et al. 2009). Significant differences in AMF abundance in EFBs at the genus level between the four groups were detected using ANOVA (P < 0.05). The linear discriminant analysis effect method was used to determine differences in AMF community composition between the four groups (Segata et al. 2011). This method was carried out with default parameters in the online Galaxy tool, which was developed by Huttnerower’s group (http://huttenhower.sph.harvard.edu/galaxy/). A redundancy analysis was conducted to determine the multivariate relationship between AMF community and environmental factors using Canoco software (version 4.5, Centre for Biometry, Wageningen, The Netherlands). The effect and significance of each environmental factor on the AMF communities were evaluated using forward selection and the Monte Carlo permutation test (499 replicates).

**Results**

**AMF colonization in the roots of aquatic plants inhabiting EFBs**

The F% of EA (C. generalis in Donghu Lake), EB (C. alternifolius in Donghu Lake), SA (C. generalis in Nanhui Lake), and SB (E. crassipes in Nanhui Lake) was 100, 13.33, 70, and 96.67%, respectively. The M% of EA, EB, SA, and SB was 68.83, 3.83, 9.73, and 71%, respectively, and the A% in these groups was 6.06, 0, 0, and 6.89%, respectively. Representative images of observed AMF structures are shown in Fig. 1.
Water quality

The water quality parameters of the collected samples are shown in Tables 1 and 2. The water quality parameters of Donghu Lake (EA and EB) were significantly different from those of Nanhu Lake (SA and SB), except for COD concentration ($P < 0.05$). The water quality near EA and EB was identical because samples were collected from the same EFB in Donghu Lake. The water quality near SA and SB was significantly different ($P < 0.05$) because samples were collected from two different EFBs in Nanhu Lake. TN and TP concentrations of water samples collected from the two

Table 1 COD, nitrogen and phosphorus concentrations of the sampling water

| Samplings | COD$_{Mn}$ (mg/L) | TN (mg/L) | NO$_3^-$-N (mg/L) | NH$_4^+$-N (mg/L) | NO$_2^-$-N (mg/L) | TP (mg/L) | IP (mg/L) |
|-----------|------------------|-----------|-------------------|------------------|------------------|-----------|----------|
| EA        | 4.72 (0.75) a    | 11.35 (0.05) a | 0.56 (0.02) c    | 9.28 (0.11) a    | 0.12 (0.00) a    | 0.77 (0.12) a | 0.72 (0.01) a |
| EB        | 4.72 (0.75) a    | 11.35 (0.05) a | 0.56 (0.02) c    | 9.28 (0.11) a    | 0.12 (0.00) a    | 0.77 (0.12) a | 0.72 (0.01) a |
| SA        | 5.70 (0.92) a    | 4.37 (0.26) b  | 1.37 (0.00) b    | 2.01 (0.11) c    | 0.06 (0.00) c    | 0.09 (0.00) b | 0.10 (0.01) b |
| SB        | 4.79 (0.72) a    | 3.96 (0.13) c  | 2.10 (0.16) a    | 2.51 (0.40) b    | 0.09 (0.00) b    | 0.09 (0.00) b | 0.03 (0.03) c |

EA, *Canna generalis* collected from Donghu Lake; EB, *Cyperus alternifolius* collected from Donghu Lake; SA, *Canna generalis* collected from Nanhu Lake; SB, *Eichhornia crassipes* collected from Nanhu Lake. COD$_{Mn}$, chemical oxygen demand; TN, total nitrogen; NH$_4^+$-N, ammonia nitrogen; NO$_3^-$-N, nitrate nitrogen; NO$_2^-$-N, nitrite nitrogen; TP, total phosphorus; IP, inorganic phosphorus. Data are means (S.D.) from three replicate samples; letters show significant differences between samples according to Duncan’s test ($P < 0.05$)
lakes greatly exceeded the minimum criteria for eutrophication (TN > 0.2–0.3 mg/L, TP > 0.01–0.02 mg/L) (Table 1). This finding indicated that the water was in a state of severe eutrophication. Water samples from EA and EB showed higher concentrations of TN, NH$_4^+$-N, NO$_3^-$-N, TP and IP than those from SA and SB (P < 0.05). SB showed the lowest TN and IP concentrations, while SA had the lowest NH$_4^+$-N and NO$_3^-$-N concentrations. EA and EB showed the highest values of temperature, electrical conductivity, total dissolved solids, specific conductance, and salinity, and the lowest values of pH, redox potential, and dissolved oxygen (Table 2).

### AMF communities in EFBs

#### Overall MiSeq sequencing information

To characterize AMF communities living in the roots of aquatic plants from the three EFBs, 12 root samples of the three aquatic plant species were sequenced on an Illumina MiSeq device. A total of 650,793 clear sequences were obtained and clustered into 1087 OTUs at the 97% sequence similarity level.

#### AMF community richness and species accumulation curve analysis

After removing the singletons, the total number of Glomomycota sequences obtained from the Illumina sequencing reads was estimated to be 348,799 (53.60% of all sequences, 229 OTUs) (Supplemental Table S1). Of these, 333,963 sequences (96.50% of all Glomomycota sequences, 218 OTUs) were classified into 19 VTs of the MaarjAM database. The remaining 12,216 sequences (3.50% of all Glomomycota sequences, 11 OTUs) could not be classified into groups of known sequences represented in the MaarjAM database, but two AMF taxa (Glomus sp. MH559174.1, Archaeospora sp. MH559177.1) were found in the NCBI database. The VT numbers assigned against the MaarjAM database using data with singletons were same as those using data without singletons (Supplemental Table S2). The composition of AMF with higher than 1% relative abundance in each community is shown in Supplemental Table S3.

The species accumulation curve of AMF living in aquatic plant roots from the three EFBs reached an asymptote (Supplemental Fig. S2). This finding indicated that the sampling intensity was adequate, and this was also supported by the curve of AMF data with singletons (Supplemental Fig. S3).

As shown in Fig. 2a and b, 71% (246,763) of the sequences and 83% (190) of the OTUs belonged to Glomus. A total of 28% (99,288) of the sequences and 15% (34) of the OTUs belonged to Acaulospora, and 6.31e−3% (22) of the sequences and 1% (2) of the OTUs belonged to Claroideoglomus. Furthermore, 1% (2726) of the sequences and 1% (3) of the OTUs belonged to Archaeospora. Therefore, most of the AMF species obtained from the three EFBs belonged to Glomus, followed by Acaulospora. The proportional distribution of AMF data with singletons was similar to these results (Supplemental Fig. S4).

#### AMF assemblage in the different samples

Glomus was the dominant genus in all groups except SB in which the dominant genus was Acaulospora (63.05%) (Fig. 2c). Glomus was the dominant genus in all root samples, except for SB2, which was dominated by Acaulospora (99.94%) (Fig. 2d). The same results were obtained with the database with singletons (Supplemental Figs. S5 and S6).

The number of Glomomycota sequences ranged from 14 (EB1) to 57,551 (SB2), forming 6 (SA1) to 103 (EA2) OTUs and 3 (SA3, EB2) to 15 (SA2) VTs (Table 3). Similar results were obtained for the data with singletons (Supplemental Table S4). The total numbers of AMF sequences, OTUs, and VTs identified in EB1 were 14, 8, and 4, respectively, while they were 31, 8, and 5 in EB3, respectively (Table 3). AMF communities had a low abundance but high diversity in EB1 and EB3, and the low abundance was not attributed to sequencing errors (Supplemental Table S5). EB2 showed a much higher AMF abundance compared with EB1 and EB3 and had a dominant OTU, which accounted for 92% of the sequences (OTU397, 43,748 sequences, assigned as Glomus LES06 VTX00310). SA3 had a higher number

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**Table 2** On-line parameters of the sampling water measured in situ

| Samplings | T (°C) | pH   | EC (μs/cm) | TDS (g/L) | ORP (mv) | SC (μs/cm) | S (PPT) | AT (mmHg) | DO (mg/L) |
|-----------|-------|------|------------|-----------|----------|------------|---------|-----------|-----------|
| EA        | 7.6   | 7.53 | 334.4      | 324.35    | 110.2    | 499        | 0.24    | 764.2     | 4.44      |
| EB        | 7.6   | 7.53 | 334.4      | 324.35    | 110.2    | 499        | 0.24    | 764.2     | 4.44      |
| SA        | 7.0   | 7.69 | 310.9      | 306.8     | 130.5    | 472.8      | 0.23    | 764       | 10.98     |
| SB        | 7.3   | 7.87 | 313.7      | 307.45    | 185      | 473.1      | 0.23    | 764       | 11.35     |

EA, Canna generalis collected from Donghu Lake; EB, Cyperus alternifolius collected from Donghu Lake; SA, Canna generalis collected from Nanhu Lake; SB, Eichhornia crassipes collected from Nanhu Lake. T, temperature; EC, electrical conductivity; TDS, total dissolved solids; ORP, redox potential; SC, specific conductance; S, salinity; AT, atmosphere; DO, dissolved oxygen.
Fig. 2 Pie graphs show the proportional distributions of OTUs (a) and sequences (b) in the *Glomeromycota* phylum detected in three EFBs. Histograms show the proportional distributions of sequences at the genus level detected in four groups (c) and 12 samples (d).

Table 3 Samples, number of obtained sequences (total, assigned to known *Glomeromycota* taxa), Chao index, and number of detected AMF taxa

| Samplings | Total sequences | Known AMF sequences | No. of known AMF OTUs | Chao index | No. of VTs | No. of taxa in NCBI |
|-----------|-----------------|---------------------|-----------------------|------------|------------|-------------------|
| Remove singleton |                  |                     |                       |            |            |                   |
| EA1       | 54,345          | 43,764              | 85                    | 96.00      | 4          | *Glomus* sp.     |
| EA2       | 53,995          | 41,684              | 103                   | 114.14     | 5          | *Glomus* sp.     |
| EA3       | 61,880          | 52,490              | 91                    | 108.27     | 4          | *Archaeospora* sp., *Glomus* sp. |
| EB1       | 57,115          | 14                  | 8                     | 10.00      | 4          |                   |
| EB2       | 53,041          | 47,557              | 39                    | 46.00      | 3          | *Archaeospora* sp. |
| EB3       | 49,672          | 31                  | 8                     | 18.00      | 5          | *Archaeospora* sp. |
| SA1       | 55,549          | 108                 | 6                     | 9.00       | 4          |                   |
| SA2       | 49,054          | 278                 | 21                    | 23.50      | 15         | *Glomus* sp.     |
| SA3       | 48,025          | 5255                | 10                    | 11.50      | 3          | *Glomus* sp.     |
| SB1       | 47,035          | 44,880              | 43                    | 46.00      | 5          | *Glomus* sp.     |
| SB2       | 61,121          | 57,551              | 43                    | 48.14      | 8          |                   |
| SB3       | 59,961          | 55,187              | 90                    | 93.11      | 9          | *Archaeospora* sp. |
| Total     | 650,793         | 348,799             | 229                   | 19         | 2          |                   |
of sequences compared with SA1 and SA2 because of the dominant OTU914 (5064 sequences). The OTU914 had no hits in the MaarjAM database, but was assigned as Glomus sp. in the NCBI database (MH559174.1). SA2 had a significantly higher number of OTUs and VTs compared with SA1 and SA3.

EA1, EA2, and EA3 shared four common VTs (Glomus VTX00113, Glomus VTX00114, Glomus VTX310, and Glomus VTX419), and one common NCBI taxon (Glomus sp. MH559174.1), representing 100, 83, and 83% of their total taxa, respectively (Supplemental Fig. S7). EB1, EB2, and EB3 shared three common VTs (Acaulospora VTX00028, Glomus VTX00310, and Glomus VTX00114), representing 75, 75, and 50% of their total taxa, respectively. SA1, SA2, and SA3 shared two common VTs (Glomus VTX00114 and Acaulospora VTX00028), representing 50, 12.5, and 50% of their total taxa, respectively. SB1, SB2, and SB3 shared five common VTs (Acaulospora VTX00028, Acaulospora VTX00029, Glomus VTX00093, Glomus VTX00114, and Glomus VTX00419), representing 83%, 62.5%, and 50% of their total taxa, respectively.

EA, EB, SA, and SB shared four common VTs (Glomus VTX00114, Glomus VTX00113, Glomus VTX00310, and Glomus VTX00419), representing 57, 57, 21, and 36% of their total VTs, respectively (Supplemental Fig. S8). No single VT was common to all 12 samples. Metastats analysis (Table 4) showed a significant difference in the abundance of Glomus between EA and EB ($P < 0.05$). Significant differences were also observed in the abundance of Glomus and Acaulospora between SA and SB (both $P < 0.05$), in the abundance of Glomus and Acaulospora between EA and SB (both $P < 0.05$), and in the abundance of Glomus and Acaulospora between EB and SB (both $P < 0.05$). Similar Metastats analysis results for data with singletons are shown in Supplemental Table S6.

Non-metric multidimensional scaling ordinations of unweighted UniFrac compositional dissimilarities and permutational ANOVA results indicated that AMF communities in the three EFBs could be significantly separated with a $P$ value of 0.002 (Fig. 3). AMF communities for data with singletons in the three EFBs could also be significantly separated with a $P$ value of 0.003 (Supplemental Fig. S9).

AMF communities showed significantly different abundances of Diversisporales, Acaulosporaceae, Acaulospora, Glomerales, Glomerales, and Glomus between the four groups ($P < 0.05$) (Fig. 4a). The relative abundance of Acaulospora was highest in SB and lowest in EB (Fig. 4b), while that of Glomus was highest in EA and lowest in SB (Fig. 4c).

**Distribution of taxonomic abundance**

We used the index of Akaike Information Criterion (AIC) to differentiate between the fit of several theoretical taxonomic abundance distribution models to the whole (12 samples together) data. We found that the lognormal model fit the observed data better (AIC = 112,516) than the niche preemption (geometric series) model (AIC = 212,343), Zipf model (AIC = 212,004), and broken stick model (AIC = 1,331,638) (Fig. 5). Among the four groups, EA and SB showed the best fit in the lognormal model, but EB and SA showed the best fit in the Zipf model (Supplemental Fig. S10). Similar results were obtained for the data with singletons (Supplemental Figs. S11 and S12). SHE analysis showed that $\ln(S)$ and $\ln(H^*)$ slowly increased as the number of sequences increased, while $\ln(E_{BG})$ slowly declined to become flat and $\ln(E_{BG})/\ln(S)$ remained flat as the number of sequences increased (Supplemental Fig. S13). As reported by Magurran (2004), this particular pattern is a characteristic of a lognormal species abundance distribution (SAD). On the basis of our results, we concluded that the distribution of AMF abundance in the three EFBs was lognormal. Similar SHE analysis results were obtained for the data with singletons (Supplemental Fig. S14).

**Overdominance and inequitability in AMF assemblage**

The most abundant taxon OTU393 (Acaulospora Alguacil10 Aca2 VTX00028) was more than 35 times ($O = 35.18$) as abundant as expected in an even community, and 89% of the OTUs were less abundant than expected in such a perfectly even community ($I = 0.89$) (Supplemental Fig. S15).

**Table 4** Metastats analysis for the AMF abundance without singletons at the genus levels in the roots of aquatic plants from the three EFBs based on Illumina Miseq sequencing data

| Taxon    | EA vs. EB ($P$-value/Q-value) | SA vs. SB ($P$-value/Q-value) | EA vs. SA ($P$-value/Q-value) | EA vs. SB ($P$-value/Q-value) | EB vs. SA ($P$-value/Q-value) | EB vs. SB ($P$-value/Q-value) |
|----------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Glomus   | 0/0                           | 0.03/0.15                     | --                            | 0.03/0.16                     | --                            | 0.04/0.36                     |
| Acaulospora | --                           | 0/0                           | --                            | 0/0                           | --                            | 0/0                           |
| Archaeospora | --                           | --                            | --                            | --                            | --                            | --                            |
| Claroideoglomus | --                           | --                            | --                            | --                            | --                            | --                            |

"--" represents the $P$-value $> 0.05$ and data now shown here.
To assess the importance of the most abundant OTU in the AMF communities inhabiting the three EFBs, the abundance ratio of the most abundant OTU to the second most abundant OTU (1:2) was calculated to be 1.2. Additionally, the ratio of the most abundant OTU to the third most abundant OTU (1:3) was calculated to be 1.6. The O of the four groups ranged from 21.24 (EA) to 39.45 (EB). In EA, the relative abundance of the most dominant AMF taxon OTU960 (Glomus irregulare VTX00114) was 17% and the richness was 23,600. In EB, the relative abundance of the most dominant AMF taxon OTU397 (Glomus LES06 VTX00310) was 92% and the richness was 43,768 (Supplemental Fig. S16).

Relationships between the AMF community and environmental variables

Redundancy analysis (Fig. 6) showed that different environmental variables had differential effects on the AMF community. The eigenvalues of axes 1 and 2 were 0.4222 and 0.2105, explaining 42.22% and 21.05%, respectively, of the variance in the AMF–environment relationship. TN, TP, IP, NH4+-N, NO3−-N, dissolved oxygen, and pH had significant effects on the composition of the AMF community, among which pH was the most prominent factor. SB was located in the first quadrant, while EA, EB, and SA were located in the second quadrant. EA, EB, and part of SA (SA1 and SA3) were positively correlated with TN, TP, IP, and NH4+-N, and SB was positively correlated with pH.

Discussion

AMF colonization in the roots of aquatic plants inhabiting EFBs

Previous studies have shown that C. generalis is colonized by AMF in terrestrial and aquatic habitats (Aziz and Abd Al-Latif 2018; Calheiros et al. 2019; Dong et al. 2017; El Faiz et al. 2015), and E. crassipes can also establish mutualistic symbiosis with AMF (Bagyaraj et al. 1979; de Marins et al. 2009; Gunathilakae et al. 2018). The association between AMF and C. alternifolius in a natural aquatic environment has not been previously reported.

In the present study, we analyzed the AMF colonization rates of three types of plant roots collected from Donghu Lake and Nanhu Lake. We found a high level of root AMF colonization in the three EFBs from these lakes. This finding was surprising in that such a high degree of mycorrhizal colonization occurred under long-term waterlogging. Stenlund and Charvat (1994) also reported AMF colonization in the roots of three Typha clones inhabiting floating mats located in Lake Owasso, Minnesota, and the colonization rate ranged from 4 to 13%.

We found differences in the AMF colonization of roots between the different plant species. The F% and M% showed high values in all groups except EB (C. alternifolius). C. alternifolius is a species belonging to the family Cyperaceae, which is usually thought to be a non-mycorrhizal plant.
Fig. 4 Linear discriminant analysis effect size analysis results of the AMF community in three EFBs. a Taxa with significant differences between the four groups. b Relative abundance of the *Acaulospora* genus in the four groups. c Relative abundance of the *Glomus* genus in the four groups.

Fig. 5 Statistical comparison of the fit of basic SAD models for the whole AMF community. AIC and model fits for several basic SAD models, including the broken stick (null model), preemption, lognormal, Zipf, and Zipf–Mandelbrot models. The AIC value (lower is better) indicates that the lognormal model is the best fit.
family. According to a previous study, mycorrhiza in plant species of Cyperaceae may occur in older roots, while young roots may be highly resistant to AMF (Brundrett 2009). In this study, we collected only fine young roots to analyze AMF colonization, which might have resulted in a low F% and M% in EB. The high A% of EA and SB suggested AM symbiosis in these aquatic plants. Considering that the arbuscule is an organ for exchanging resources between two partners (Smith and Read 2008), the existence of arbuscules indirectly indicates functional symbiosis. In a eutrophic waterbody, host plants rely not only on nutrients that are directly absorbed by their well-developed roots, but also on resources supplied by AMF to maintain normal physiological activities (e.g., synthesis of amino acids and enzymes, cell division, photosynthesis, and respiration). Additional nutrient uptake from AMF is accompanied by the promotion of plant growth, resulting in a greater plant height, diameter at ground level, and biomass (Hu et al. 2020; Ingraffia et al. 2019). In our study, a high M% was recorded in SB (E. crassipes), which is consistent with the results of Gunathilakae et al. (2018), de Marins et al. (2009), and Bagyaraj et al. (1979). However, our finding is different from that of Wang and Zhao (2006), who sampled roots of E. crassipes free-floating in lakes and streams, and found no mycorrhizal symbionts in the roots. E. crassipes should theoretically easily be colonized by AMF because of its well-developed aerenchyma in roots. E. crassipes can transport photosynthetically produced oxygen through the aerenchyma to the root tips, consequently providing a basic survival condition for the aerobic metabolism of AMF (Tanner and Clayton 1985). The difference between our study and Wang and Zhao (2006) might be related to different methods of AMF colonization analysis (different decolorization and dyeing processes). However, even in the same plant species, we found that the F% and M% were lower in SA (C. generalis collected from Nanhu Lake) than in EA (C. generalis collected from Donghu Lake). This difference between the groups might be due to differences in environmental factors, especially phosphorus concentrations, because the phosphorus concentration of EA was significantly higher than that of SA (Table 1). Phosphorus concentrations in the rhizosphere are considered one of the main abiotic factors that affect AMF colonization of aquatic plants (Tang et al. 2001; Wang et al. 2009; White and Charvat 1999; Xie et al. 2014). Wang et al. (2009) suggested a “bell-shaped” relationship between AMF colonization and soil phosphorus concentrations. In this relationship, when soil phosphorus concentrations are high or low enough, AMF colonization is inhibited, which can also occur in aquatic ecosystems.

**AMF communities in the roots of aquatic plants inhabiting EFBs**

AMF communities in EFBs have only been investigated in a study by Stenlund and Charvat (1994) using a morphological method. These authors found a low abundance of AMF in EFBs, and the identified AMF communities were dominated by T. augustifolia (average rate: 4–5%), T. xglauca (average rate: 4–5%), and T. latifolia (average 13%). These AMF communities also included G. albidum, Funneliformis caledonium, Claroideoglomus etunicatum, and G. microcarpum. Both the diversity and abundance of AMF in EFBs in our study were higher than those reported by Stenlund and Charvat (1994). This difference between studies may be due to a different resolution and depth of identification. In previous studies, the distribution of AMF in aquatic ecosystems was extensively investigated by morphological methods. Numerous AMF genera were identified, including Glomus, Acaulospora, Claroideoglomus, Funneliformis, Rhizophagus, Scutellospora, Racocetra, Ambispora, Dentiscutata, and Gigaasporea, and Glomeraceae was the dominant AMF family (Choudhury et al. 2010; Miller and Bever 1999; Radhika and Rodrigues 2007; Wang et al. 2009). Molecular approaches, including restriction fragment length polymorphism, denaturing gradient gel electrophoresis, temporal temperature gradient gel electrophoresis, clone libraries, and single-stranded conformation polymorphism, have also been applied to investigate the AMF communities in aquatic ecosystems (Calheiros et al. 2019; Guo and Gong 2014; Likar et al. 2009; Nielsen et al. 2004; Wolfe et al. 2007). Consistently, results from these approaches indicated that the dominant AMF family was Glomeraceae. Although
morphological and molecular methods are traditional and effective, the obtained results are partial and one-sided because of their incompleteness and inaccuracy in detecting AMF taxa with a low abundance. NGS methods have shown an increasing amount of AMF abundance in aquatic habitats. Forty-seven AMF VTs from seven families (Glomeraceae, Archaeosporaceae, Acaulosporaceae, Gigasporaceae, Claroideoglomeraceae, Diversisporaceae, and Paraglomeraceae) have been found in roots of the submerged aquatic plant Lobelia dortmanna, and the dominant AMF family was Glomeraceae, followed by Archaeosporaceae and Acaulosporaceae (Moora et al. 2016). Ban et al. (2017) investigated AMF communities in the roots of Phragmites australis inhabiting wetlands polluted by three heavy metals. These authors obtained 258 OTUs from 235,213 sequences affiliated with 6 Glomeromyota families, among which Glomeraceae and Paraglomeraceae were the most dominant and second most dominant families, respectively. Xu et al. (2018) investigated the distribution of AMF in the roots of P. australis from two vertical-flow constructed wetlands (VFCCWs) and obtained a total of 33,031 AMF sequences. These sequences were assigned to 54 OTUs and 17 VTs from Glomus, Claroideoglomus, Acaulospora, and Gigaspora. Compared with the abovementioned studies that used the NGS method, the AMF diversity in our study was similar to that found by Moora et al. (2016) and Ban et al. (2017), but higher than that reported by Xu et al. (2018). This difference between studies might be because the samples in Xu et al. (2018) were collected from a non-natural environment and the origin of AMF was single (inoculated only with Rhizophagus intraradices inoculum).

Our study showed that AMF living in the investigated EFBs belonged to four Glomeromycota genera (Glomus, Acaulospora, Claroideoglomus, and Archaeospora). Glomus was the dominant AMF genus, and Glomeraceae was the dominant AMF family. These results are consistent with most studies that used morphological, molecular, and NGS methods. Different aquatic plant species showed different abundances (14–57,551 sequences), diversity (6–103 OTUs, 3–15 VTs), and community composition of AMF, which might be related to the different host plant species. Indeed, according to Su et al. (2011), AMF had a strong host species preference, and the host plant species was a main factor influencing the spore density, species richness, and diversity of AMF. The reason for this finding is that plants can regulate carbon allocation to roots, produce secondary metabolites, and change soil environmental conditions.

C. generalis is a common mycorrhizal plant species in terrestrial and aquatic habitats (Aziz and Abd Al-Latif 2018; Calheiros et al. 2019; Dong et al. 2017; El Faiz et al. 2015). Calheiros et al. (2019) used the denaturing gradient gel electrophoresis method to analyze AMF communities in the roots of C. generalis, C. flaccida, and Watsonia borbonica inhabiting a horizontal subsurface flow constructed wetland. They showed that Glomus was the dominant AMF genus, which is consistent with our finding. However, in a study by Calheiros et al. (2019), the roots of C. generalis harbored a low diversity of AMF, which belonged to only Glomus sp. and Acaulospora sp. In this study, AMF were found in the roots of C. generalis (EA and SA) and belonged not only to Glomus and Acaulospora but also to Claroideoglomus and Archaeospora. We cannot rule out the possibility of the existence of other AMF genera because of limitations of resolution and depth of identification as shown by Calheiros et al. (2019). Additionally, although EFBs and constructed wetlands (CWs) are aquatic ecosystems, the type of substrate in EFBs is different from that in CWs, and the content of substrate used in EFBs is lower, which may affect AMF diversity. Cyperaceae is generally considered a non-mycorrhizal plant family; however, occasional arbuscules have been observed in the roots of sedges by some investigators (Harley and Harley 1987; Muthukumar et al. 2004), which has led some authors to propose a possible classification of Cyperaceae as a variable mycorrhizal plant family (Brundrett 2009). However, other researchers prefer to attribute the occasional reports of AM in Cyperaceae to an error in sampling, assessment, and diagnoses or to non-functional arbuscules (Brundrett 2009). In recent years, an increasing number of investigations have confirmed that several plant species from the Cyperaceae family are colonized by AMF (Veselkin et al. 2014), even in aquatic ecosystems (Fusconi and Mucciarelli 2018). In our study, C. alternifolius (Cyperaceae) in EB1 and EB3 showed a low AMF abundance with 14 and 31 sequences, respectively, but a relatively high diversity, with 8 OTUs and 4 VTs in EB1, and 8 OTUs and 5 VTs in EB3. However, C. alternifolius in EB2 showed a high abundance (47,557) and diversity (39 OTUs). The AMF community found in the roots of C. alternifolius indicates that NGS techniques are effective for identifying rare species. The higher accurate identification ability of NGS techniques compared with conventional morphological identification is attributable to its higher resolution. We observed AMF colonization in the roots of C. alternifolius in this study. Taking into account previous investigations on mycorrhizal fungi detected in several plant species of Cyperaceae (Fusconi and Mucciarelli 2018; Harley and Harley 1987; Lagrange et al. 2013; Muthukumar et al. 2004; Veselkin et al. 2014), we speculate that Cyperaceae is not a non-mycorrhizal plant family, but a variable mycorrhizal plant family instead. The AMF abundance and diversity in the roots of Cyperaceae family plant species may depend on environment variables, especially phosphate (P) concentrations. Lagrange et al. (2013) reported a significant positive correlation between mycorrhizal colonization in Costularia comosa and soil P concentrations, suggesting a functional symbiosis between them. Because of
limitations of identification techniques, researchers might not find AMF in some plant species of Cyperaceae and then consider them to be non-mycorrhizal plants. With the rapid development and popularization of NGS technology, the distribution of AMF communities in the roots of mycorrhizal plants is likely be more accurately investigated, and even detected in some plant species that were originally considered non-mycorrhizal.

AMF is widespread in terrestrial ecosystems (Ciccolini et al. 2016; De Beenhouwer et al. 2014; Rodríguez-Echeverría et al. 2017; Sun et al. 2016). Almost without exception, Glomeraceae is the dominant AMF family in most terrestrial environments, which is in accordance with our finding in the aquatic ecosystem. However, there are some differences between terrestrial and aquatic ecosystems. Diversisporaceae, Claroideoglomeraceae, and Paraglomeraceae have been reported as the second most dominant AMF families in most terrestrial ecosystems (Ciccolini et al. 2016; De Beenhouwer et al. 2014; Rodríguez-Echeverría et al. 2017), but in our study, Acaulosporaceae was the second most dominant family in an aquatic ecosystem. Acaulosporaceae is a common family in aquatic ecosystems. Yang et al. (2016) found that the relative abundance of OTUs belonging to the genus Acaulospora in Populus deltoides was significantly increased after short-term waterlogging. Previous studies have also shown that Acaulospora is a common genus in the roots of aquatic plants (Baar et al. 2011; Calheiros et al. 2019; Nielsen et al. 2004; Wang et al. 2016; Wirsel 2004). Additionally, the numbers of sequences and OTUs that we obtained in this study were not less than those in most terrestrial studies, and much more than those in several terrestrial studies. In conclusion, the three EFBs in this study harbored diverse and abundant AMF, and the AMF communities in EFBs were different from those in terrestrial ecosystems. These findings indicated a unique distribution of AMF in these EFBs.

**Taxonomic abundance distribution**

SAD, which incorporates species richness and abundance information, can reflect comprehensive information of a microbial community. In this study, the AIC values showed that the SAD of AMF inhabiting the three EFBs (12 samples together) fit the lognormal model best. AMF in different natural environments show different SAD types, and might fit the lognormal, broken stick, or niche preemption model (Ban et al. 2017; Dumbrell et al. 2010; Unterseher et al. 2011). Our finding of SAD is consistent with previous observations (Dumbrell et al. 2010; Moebius-Clune et al. 2013; Nielsen et al. 2016; Unterseher et al. 2011). Among the four groups, EA and SB showed the best fit with the lognormal model, but EB and SA showed the best fit with the Zipf model. The different results of the EB and SA groups can be attributed to the most abundant OTU397 (Glomus LES06 VTX00310) in EB and the most abundant OTU914 (Glomus sp. MH559174.1) in SA. There were absolutely dominant OTUs in these two groups, which is also depicted in Supplemental Fig. S16.

The differences found between the four groups might also be related to complex environmental factors, which shaped the AMF community composition and abundance distribution. Redundancy analysis results (Fig. 6) showed that AMF community composition was closely related to nutrient concentrations (nitrogen and phosphorus) in the two eutrophic lakes, which is consistent with previous studies (Guo and Gong 2014; Wang et al. 2020; Xiang et al. 2014). AMF promotes the absorption of nitrogen and Pi by plants. After being taken up through ammonium transport proteins, nitrate transport proteins, and Pi transport proteins in the extraradical mycelium, inorganic nitrogen and Pi are incorporated into amino acids and ATP, respectively. Nitrogen and Pi are then translocated from the extraradical mycelium to the intraradical mycelium as arginine and polyphosphate, respectively. Ammonia and Pi are released from arginine and polyphosphate breakdown and eventually transported into the host root cells (Govindaraju et al. 2005; Javot et al. 2007; Kikuchi et al. 2016). The AMF community composition was positively correlated with TN and TP in most groups. This finding suggests that this mutualistic symbiosis played an important role in eutrophic water and also affected both host plants and AMF in forming mycorrhizal symbionts. The correlations between AMF communities and environmental factors were different in different groups. The reason for this finding may be that different genera of AMF have different adaptabilities to an ecological environment. Not only unilateral effects of environmental factors but also niche differentiation caused by host and interspecific interaction will then result in different SAD types of AMF communities.

This study was conducted at a small spatial and temporal scale, and therefore, the SAD types might have been scale-dependent. AMF SAD on a landscape or continental scale might be determined by their biogeography, species range size, and the interaction between locally abundant taxa and widespread generalist species (Dumbrell et al. 2010).

**Overdominance and inequitability**

Among the total abundance of AMF communities examined across the three EFBs, the dominant AMF taxon (OTU393, Acaulospora Alguacil10 Aca2 VTX00028) accounted for 16%. Similar to our finding, Fierer et al. (2007) analyzed bacterial communities in the soil and rhizosphere, and found that the most dominant bacterial OTU accounted for 18 to 26% of the total abundance. A similar level was also observed in phytoplankton communities (Venrick 1990).
However, in the four groups in this study, the dominant AMF taxon occupied 17% (EA: OTU960, *Glomus irregularare* VTX00114), 92% (EB: OTU397, *Glomus* LES06 VTX00310), 90% (SA: OTU914, *Glomus* sp.), and 35% (SB: OTU393, *Acaulospora* Algaucil10 Aca2 VTX00028) of the total abundance. This finding is similar to that obtained by Dumbrell et al. (2010). Additionally, the dominant species of AMF differed among the four groups, which indicates that the role of stochastic events in producing a community was heavily dominated by one or two species that had an unusually high recruitment rate by chance (Poulin et al. 2008).

**Application potential of AMF in the remediation of water environment**

In aquatic and wetland habitats, the functions of AMF have not been extensively studied. AMF affect the composition, succession, and diversity of the plant community (Wang et al. 2009; Zhang et al. 2014); promote plant growth and the ability of water plants to adapt to the eutrophic environment, become mycorrhizal symbionts with aquatic plant roots, and proliferate in large numbers, becoming the dominant AMF. At the same time, AMF provide benefits to the host plants, especially when the host plants are under various stressful conditions. Although we are unsure what these actual benefits are, the rich diversity and high abundance of AMF in EFBs suggest that their application in plant-based bioremediation of wastewater has great potential.

In summary, the roots of *C. generalis*, *C. alternifolius*, and *E. crassipes* inhabiting the three EFBs harbored diverse AMF, which belonged to four Glomeromycota genera (*Glomus*, *Acaulospora*, *Claroideoglomus*, and *Arachaeospora*). *Glomus* and *Acaulospora* were the most dominant and second most dominant genera, respectively. The number of sequences and OTUs that we obtained in this study exceeded most of the records in terrestrial ecosystems, which indicates the presence of diverse and abundant AMF communities in EFBs. Different aquatic plant species showed varying degrees of AMF colonization, diversity, and abundance. Significant differences were found at different taxonomic levels between root samples of different groups. These differences might be attributable to complex environmental factors and strong microbial interspecies interactions in these habitats. The AMF community composition was closely related to nutrient concentrations (nitrogen and phosphorus), indicating that functional mycorrhizal symbiosis may act on plant-based bioremediation of wastewater. Greater attention should be paid to the distribution and function of AMF in aquatic ecosystems in future studies.

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files. The raw reads have been deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA550311).

Declarations

Conflict of interest The authors declare no competing interests.

Consent to participate A signed consent was taken from all the participants.

Consent for publication Written informed consent for publication was obtained from all participants.

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