Bacterial communities in co-cultured fish intestines and rice field soil irrigated with aquaculture wastewater

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Abstract
In some regions, integrated rice-fish farms have been developed to balance the needs of aquaculture wastewater discharge and rice field irrigation. In this type of aqua-agriculture system, soil is irrigated with aquaculture wastewater, and intestinal bacteria in cultured fish species likely impact soil bacteria through irrigation. However, little is known about the relationship between soil bacteria and intestinal bacteria in some carp species commonly co-cultured in some Asian regions. Therefore, we co-cultured five carp species in aquaculture ponds and used the aquaculture wastewater to irrigate rice fields for over 5 years, and then compared carp intestinal bacterial communities with rice field soil bacterial communities. The results from analysis of similarity and SourceTracker analysis showed that a low similarity ($R = 0.7908$, $P = 0.001$) and contribution (an average of 9.9% of bacterial genera) of intestinal bacteria to soil bacterial communities although 77.5% of soil bacterial genera were shared by intestinal bacteria. Our results also indicated that intestinal bacteria in the numerically dominant fish species in the co-culture system do not necessarily impact soil bacteria more significantly than those of less abundant carp species, and that intestinal bacterial communities in one single fish species may impact certain soil bacterial phyla more significantly than others. Our results provide a better understanding of the impact of aquaculture wastewater on rice fields and will be helpful for the development of this type of aqua-agriculture system.

Keywords: Asian carp, Bacterial community, Integrated rice-fish system, Intestinal bacteria, Soil bacteria

Introduction
Fish are an important protein resource for humans. In recent years, population growth has led to an increased demand for fish, which has resulted in overfishing and the decline of wild fish stocks (Yang et al. 2019). Aquaculture production has increased to alleviate the increasing pressure of fish demand. Zhang et al. (2022) reported that freshwater finfish culture dominates global aquaculture production, and they predicted that aquaculture will continue to depend more on land-based systems than on those in the sea.

Carp species, such as black carp (Mylopharyngodon piceus), grass carp (Ctenopharyngodon idella), silver carp (Hypophthalmichthys molitrix), bighead carp (Hypophthalmichthys nobilis), common carp (Cyprinus carpio), and crucian carp (Carassius auratus), belong to the family Cyprinidae. Some of these carp species have been cultured in Asia for centuries (Nakajima et al. 2019) and account for a large proportion of freshwater aquaculture production (Phelps et al. 2017; Li et al. 2021a). Traditionally, carp species are co-cultured in the same ponds to optimize the use of feed and space by integrating multiple trophic levels (Wang et al. 2016; Li et al. 2021a), and high-density co-culture of these carp species has been developed in many regions.

At the same time, concerns about the ecological consequences of aquaculture wastewater discharge are...
growing. Aquaculture practices frequently lead to water pollution (Grabicova et al. 2020; Han et al. 2020; Ta and Babel 2020), particularly that caused by nitrogenous compounds generated by feed and fish feces (Qi et al. 2019; Kim et al. 2020). Aquaculture wastewater discharge frequently causes eutrophication in receiving water bodies. In some regions, purification and reuse of aquaculture wastewater in aquaculture farms is mandatory and direct discharge into natural waters is prohibited.

Many techniques have been developed to combat aquaculture pollution (Zadinelo et al. 2018; Choi et al. 2020). For example, aquaculture wastewater has been used to irrigate agricultural crops in some aquaculture-agriculture complexes. Rice (Oryza sativa) is a major global food crop and requires a huge amount of water to grow. Approximately 30 million hectares of rice fields are cultivated in China, and the use of aquaculture wastewater in rice fields is widespread in some regions of northwest China, also partly because of limited water resources.

Soil bacteria play a crucial role in the soil ecosystem, and their diversity and community structure have been widely studied in different rice field environments (Chen et al. 2017; Huang et al. 2020; Li et al. 2021b). They transform soil structure, decompose organic matter, circulate soil nutrients (Baldrain 2019; Kumar et al. 2019; Hermans et al. 2020), and support plant growth (Garbeva et al. 2004; Tartaglia et al. 2020; Van Tung et al. 2021). Soil bacterial community structure can also be an indicator of the quality of the soil ecosystem and of the soil itself (Hermans et al. 2020). Many studies have also focused on bacterial communities in different aquaculture systems (Martins et al. 2013; Chang et al. 2019; Li et al. 2022b). Bacteria in fish intestines were found to be important in digestion and immunity of host animals (Cabello et al. 2020; Neissi et al. 2020) by producing enzymes that can decompose food and release many kinds of essential and beneficial biological substances (Rurangwa and Verdegem 2015) and by suppressing pathogen growth via antagonistic effects and mucosal protection (De Schryver and Vadstein 2014). Intestinal bacterial communities in different species of Asian carp have been reported in a number of studies (Ni et al. 2021; Yu et al. 2021).

When irrigating rice fields with aquaculture wastewater, fish feces carrying intestinal bacteria may be brought into the fields. Several researchers previously reported that fish culture and irrigation using culture wastewater significantly impacted soil bacterial communities in rice fields (Chen et al. 2017; Zhao et al. 2021). However, direct comparisons of intestinal bacterial communities in co-culture fish species and soil bacteria in rice fields irrigated with aquaculture wastewater have not been reported to date. Which fish species in the co-culture system impacted the soil bacterial communities more significantly or contributed more to the impacts of aquaculture wastewater on the soil bacterial communities? Are the dominant fish species certainly impact soil more significantly or it depends on the species-specificity? One fish species impacted all soil bacterial phyla to the same extent?

In this study, we compared intestinal bacterial communities in five co-cultured carp species with soil bacterial communities in rice fields irrigated with aquaculture wastewater for over 5 years. The results of this study can provide a better understanding of the impact of aquaculture wastewater irrigation on rice field soil in a rice-fish system.

Materials and methods

Experimental area

One rice-fish farm located in the northwest of China (106.36°E, 38.62°N) was used in this study. Thirty ha² of aquaculture ponds were used to co-culture five Asian carp species (grass carp, common carp, silver carp, crucian carp, and bighead carp). Additionally, 50 ha² of fields were used to grow rice, and they were irrigated with aquaculture wastewater for more than 5 years. The fish stocking density was 15–20 thousand individuals per ha². A single artificial compound feed was used in this farm, consisting of 28% crude protein, 18% crude ash, 9% crude fiber, and 4% crude fat. In this study, we collected fish from five aquaculture ponds to obtain intestinal bacteria and took water samples from four ponds and soil samples from six rice fields to obtain water and soil bacteria. Details about the sampling sites are provided in the Supporting Information (Additional file 1: Figure S1 and Additional file 4: Table S1).

Sample collection

Samples were collected in August of 2020. In the month of sampling, this study region is sunny and dry, and the daily maximum and minimum air temperatures are around 30 °C and 18 °C, respectively. Three individuals of each fish species from each pond were randomly chosen to collect the intestinal contents. The length, weight, and height of each fish were measured. The fish were rapidly killed and the intestinal tract from the stomach or fore-intestine to the anus, excluding the stomach or fore-intestine, was removed using a sterile surgical lancet and scissors. The intestinal contents of each fish were then squeezed into sterile 50 ml centrifuge tubes using sterile tweezers (Wu et al. 2012; Sun et al. 2020), and the wet weight of the sample was measured. After being well mixed, approximately 200 mg of the intestinal contents were placed in sterile 1.5 ml centrifuge tubes and stored at—80 °C until subsequent analysis. A total of 69 intestinal samples were collected. All experiments involving
animals were performed in accordance with the protocols approved by the Animal Ethics Committee of Shanghai Ocean University (Approval ID: SHOU-DW-2020–057).

Surface water samples at 20 cm under the water surface were collected from the central points of the aquaculture ponds twice, with a 2-week interval between sampling. Immediately after sampling, the 500 ml water samples were filtered using 5-μm pore filters to remove suspended feed, feces, and planktonic algae (Zhao et al. 2017; Liu et al. 2019). Next, 0.22-μm pore filters were used to collect planktonic bacteria samples (Li et al. 2022a). All collected planktonic bacteria samples on filters were stored in sterile centrifuge tubes at—80 °C until subsequent analysis. A total of 8 planktonic samples were collected.

In each rice field, soil samples were collected from four evenly distributed sampling sites. At each sampling site, soil between rice plants was collected from the soil surface with a depth of 0—5 cm (Zhou et al. 2021). The collected soil was well mixed, and approximately 100 mg of soil were placed in sterile 1.5 ml centrifuge tubes and stored at—80 °C until subsequent analysis. A total of 24 soil samples were collected.

Bacterial community analysis
Metagenomic DNA in bacterial communities was extracted from each collected sample using the E.Z.N.A. soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA). Using the primers 338F and 806R (Srinivasan et al. 2012) with sequencing barcodes, the V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified from extracted metagenomic DNA using an Applied Biosystems GeneAmp 9700 PCR thermocycler (Carlsbad, CA, USA). PCR amplons of the bacterial 16S rRNA gene were paired-end sequenced (2 × 300) on an Illumina MiSeq platform (San Diego, CA, USA) by a commercial company (MajorBio, Shanghai, China). Raw 16S rRNA gene sequencing reads were quality-filtered by fastp (Chen et al. 2018) and merged by FLASH (Magoc and Salzberg 2011). Operational taxonomic units (OTUs) were clustered using UPARSE (Edgar 2013). Alpha diversity indexes were calculated using Mothur (Schloss et al. 2009), and beta diversity (Bray–Curtis distance) was calculated using Qiime (Bolyen et al. 2019). The taxonomy of each OTU representative sequence was analyzed by RDP Classifier (Wang et al. 2007) against the Silva v 138 16S rRNA database (Quast et al. 2013). The raw reads of 16S rRNA gene sequences obtained in this study were submitted to the NCBI SRA database under the accession number PRJNA741343.

Statistical analysis
Statistical analysis was carried out using R version 3.3.1 (The R Foundation, Vienna, Austria). We compared the diversity indexes and bacterial abundances between sample groups using Student’s t-test and Helch’s t-test (R stats package). The numbers of core bacterial OTUs were counted using Venn diagram analysis (R Venn diagram package). The differences in community structure between sample groups were detected using analysis of similarities, non-metric multidimensional scaling analysis, and hierarchical clustering community heatmaps (R vegan package). In addition, we used SourceTracker (Knights et al. 2011) to analyze the contribution of intestinal bacteria to soil bacterial communities. Unless otherwise stated, all analyses were performed at the OTU level.

Results
Bacterial communities
The measured body parameters of 69 fish individuals and the alpha diversity data for all 101 intestinal, planktonic and soil samples are provided in the Supporting Information (Additional file 4: Table S1). The culture numbers, total body weight and intestinal contents of grass carp and crucian carp were higher than those of the other species in the culture ponds (Table 1).

After Illumina Miseq sequencing and quality controlling, 5.3 million clean sequences with an average length of 413 base pairs were obtained. After removing chloroplast sequences and conducting normalization, 27,084 sequences from each sample were used in the subsequent analysis. From the 101 libraries, a total of 13,937 OTUs and 2036 genera were obtained. Good’s coverage values of all libraries were higher than 94.5%. The dominant bacterial phyla in planktonic samples were Actinobacteria and Proteobacteria, with relative abundances of 41.3% and 30.4%, respectively. Proteobacteria (23.0%), Firmicutes (22.9%), Actinobacteria (17.8%), and Fusobacteria (12.4%) dominated intestinal samples, and Chloroflexi

| Table 1 | Body parameters of sampled fish and the yield proportion of co-cultured species in the farm |
|---------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|         | Grass carp                      | Common carp                     | Silver carp                     | Crucian carp                    | Bighead carp                    |                                |
| body weight (g)a | 902 ± 286                      | 864 ± 251                      | 1158 ± 350                     | 299 ± 103                      | 988 ± 277                      |
| intestinal content (g)a | 25.9 ± 20.8                    | 7.2 ± 7.1                      | 25.4 ± 18.3                    | 4.4 ± 3.4                      | 19.9 ± 13.9                    |
| culture number (ind)b | 121,000                        | 26,000                         | 10,000                         | 332,000                        | 9000                           |

*a* Determined based on the sampled fish

*b* The numbers of cultured fish species in the farm in 2020, which were calculated during the harvest season.
(32.4%), *Actinobacteria* (16.0%), *Proteobacteria* (12.5%), and *Firmicutes* (10.7%) dominated soil samples (Fig. 1A). The OTU table is provided in Supporting Information Additional file 5: Table S2 and the top 50 bacterial genera are provided in Additional file 2: Figure S2.

Student’s *t*-test analysis of Shannon, Simpson, Ace, and Chao indexes revealed higher diversity of the four indexes was found in soil samples than intestinal and planktonic samples (*P < 0.001*, the results of *t*-test are provided in Additional file 4: Table S1). Non-metric multidimensional scaling analysis revealed significant variations in beta diversity between intestinal, water, and soil samples, and dispersion was greater in intestinal samples than in water and soil samples (Fig. 1B) and the principal co-ordinates analysis also revealed significant variations between intestinal and soil samples (Fig. 1C). Analysis of similarity also revealed significant variations between intestinal and planktonic samples (*R = 0.3417, P = 0.002*), between planktonic and soil samples (*R = 1, P = 0.001*), and between intestinal and soil samples (*R = 0.7908, P = 0.001*). Furthermore, significant variations of beta diversity were found among the five fish species.
and soil bacterial communities were more similar with intestinal bacterial communities in common carp ($R = 0.9201, P = 0.001$) in comparison to grass carp ($R = 0.9757, P = 0.001$), silver carp ($R = 0.9771, P = 0.001$), crucian carp ($R = 0.9808, P = 0.001$) and bighead carp ($R = 1, P = 0.001$).

Core genera

The Venn diagram showed that 3596 OTUs were shared by intestinal and soil samples (Fig. 2A). Among them, 706 core OTUs were shared by intestinal, planktonic, and soil bacterial communities. Another 2890 OTUs were shared by only intestinal and soil samples, indicating that some bacterial OTUs were attached to particulate matter in the water and were filtered by the 5-μm pore filters. In total, 3596 OTUs shared by intestinal and soil bacterial communities accounted for 49.4% and 36.5% of OTUs in intestinal and soil samples. At the genus level, 513 genera were shared by intestinal, planktonic, and soil bacteria, and another 697 genera were shared by only intestinal and soil samples. In total, 1210 genera shared by intestinal and soil bacteria accounted for 72.8% and 77.5% of genera in intestinal and soil samples (Fig. 2B).

The Venn diagram also showed that 78.7%, 73.6%, 83.9%, 78.1%, and 85.6% of intestinal genera in grass carp, common carp, silver carp, crucian carp, and bighead carp were shared with soil bacterial communities, representing 58.9%, 61.7%, 33.5%, 53.8%, and 47.8% of soil genera (Additional file 3: Figure S3). The results indicated that more soil bacterial genera were shared by intestinal bacterial communities in common and grass carp. In addition, 468 genera were shared by all five fish species and 404 of them were also found in soil (Fig. 2C).

Soil bacterial phyla

The hierarchical clustering results indicated that bacterial communities in soil were more similar to that in common and grass carp than in the other species (Fig. 3A), in accordance with the comparison of Bray–Curtis distances and shared bacterial genera. For further analysis of the dominant phyla in soil, sequences allocated to Chloroflexi, Actinobacteria, Proteobacteria, and Firmicutes were retrieved from obtained libraries in this study. As an important bacterial phylum in the bacteria-plant interactions (Bahareh et al. 2021), Cyanobacteria communities were also compared.

In this study, Chloroflexi was the top bacterial phylum in the soil and significantly higher relative abundances of Chloroflexi were found in the soil than in the intestine samples. The analyses of similarity showed that soil Chloroflexi communities were more similar to that in common carp, crucian carp and grass carp than other carp species and Firmicutes communities in crucian carp, common carp and grass carp were more similar to that in soil. In addition, Actinobacteria and Proteobacteria communities in common carp and crucian carp possessed smaller Bray–Curtis distances with soil bacterial communities (Fig. 3B).

The sequences associated with Cyanobacteria were also retrieved. Helch's $t$-test analysis revealed no significant differences of Cyanobacteria abundances among planktonic, soil, and intestinal bacteria, but Cyanobacteria were more abundant in the intestinal bacterial

![Fig. 2](image-url) Venn diagrams showing the number of shared bacterial OTUs A and genera B in planktonic, intestinal and soil bacterial communities, and that the shared intestinal bacterial genera by the five carp species were also found in soil C. Fish, water and soil indicating intestinal, planktonic and soil bacterial communities.
communities in silver carp compared to the other four fish species (P < 0.001). The analyses indicated that soil Cyanobacteria communities were more similar to that in common carp and grass carp (Fig. 3B).

Source tracking
The SourceTracker analysis results showed the contributions of intestinal bacterial communities to soil (Table 2). In the soil bacterial community in rice field, an average of 9.9% of bacterial genera was confirmed from intestinal bacterial communities. The source of 3.6% of soil bacterial genera was the bighead carp intestines and 3.0% was from the crucian carp intestines. In grass carp, common carp and silver carp, intestinal bacterial communities contributed lesser bacterial genera. An average of 90.3% of soil bacterial genera was from unknown sources. Comparing between the analyzed bacterial phyla, bighead carp contributed more Cyanobacteria genera and other carp species contributed more Chloroflexi genera than other bacterial phyla. Between five carp species, grass

Table 2

| phylum           | Bighead carp % | Common carp % | Crucian carp % | Grass carp % | Silver carp % | Unknown % |
|------------------|----------------|---------------|----------------|--------------|---------------|-----------|
| bacteria         | 3.6±0.9        | 1.0±0.2       | 3.0±0.8        | 1.9±0.4      | 0.5±0.5       | 90.3±1.7  |
| Chloroflexi      | 2.3±0.7        | 2.6±0.7       | 2.4±0.8        | 3.8±0.8      | 1.5±0.5       | 87.4±2.7  |
| Actinobacteria   | 0.5±0.5        | 1.1±0.3       | 1.5±0.7        | 1.2±0.4      | 0.9±0.5       | 95.0±1.8  |
| Proteobacteria   | 1.0±0.2        | 0.9±0.3       | 1.0±0.3        | 1.0±0.0      | 0.4±0.5       | 95.8±0.8  |
| Firmicutes       | 0.9±0.3        | 0.0±0.2       | 1.3±0.6        | 0.9±0.3      | 0.3±0.5       | 96.3±0.9  |
| Cyanobacteria    | 3.0±2.4        | 0.9±0.8       | 1.0±0.7        | 0.7±0.6      | 0.7±0.6       | 93.8±4.2  |

The mean values and standard deviations were obtained from 24 soil samples.
carp contributed more Chloroflexi genera and crucian carp made more contribution of Actinobacteria and Firmicutes genera to soil bacterial communities (Table 2).

Discussion
Soil bacterial communities
Rice is an important crop species with a huge cultivated area, mainly in Asia. Traditionally, rice cultivation requires a huge amount of water. In addition, aquaculture produces wastewater that must be purified before discharge. One strategy to address these issues is to blend aquaculture operations and rice cultivation. Using wastewater from aquaculture ponds (Van Tung et al. 2021) or integrating aquaculture of suitable species in rice fields (Li et al. 2021b) improves the utilization rate of water and reduces wastewater discharge rates. Consequently, these methods have been promoted in some regions, especially in arid and semi-arid regions.

Zhao et al. (2021) reported that the soil bacterial community structure in a rice-fish system was obviously different from that of the traditional rice field, and Chen et al. (2017) detected higher bacterial community diversity in soil irrigated with aquaculture wastewater than in those irrigated with lake water. However, Li et al. (2021b) reported that the high amounts of protein entering agricultural soil via pellet feed and fish feces lowered soil bacterial diversity in a rice-fish system. In the current study, we found that bacterial diversity in rice field soil irrigated with aquaculture wastewater was lower than that reported by Chen et al. (2017) and Zhao et al. (2021), but it was higher than that of intestinal bacterial communities in the cultured fish. To date, analysis of the relationship between intestinal bacteria and soil bacteria in rice-fish systems are rare. Our results showed that 36.5% of soil OTUs and 77.5% of bacterial genera were shared by cultured carps. This result indicates that intestinal bacteria probably impact soil bacterial communities through irrigation, and that some bacterial species from the fish intestine may colonize the soil. Xiao et al. (2022) reported that microbial inoculation had a significant impact on soil bacterial community structure and an even more significant impact on rare bacteria than on dominant bacteria.

Intestinal bacterial communities
Many factors can impact intestinal bacteria, including food, drugs, and environmental conditions. Some studies have shown that most intestinal bacterial OTUs in aquatic animals are also found in the sediment and water, indicating that the intestinal bacteria in aquatic animals are derived from the environment (Sun et al. 2020). However, research has also indicated that intestinal bacterial communities are host-specific, even when various species live together in the same environment, e.g., in wild Asian carp (Li et al. 2018), in sole (Solea senegalensis) and turbot (Scophthalmus maximus) (Martins et al. 2013). In a study of grass carp, crucian carp, and bighead carp co-raised in aquaculture ponds, Li et al. (2015) found higher intestinal microbial diversity in the filter feeding bighead carp than in the other two species, which suggested that the intestinal microbiota assemblage resulted from species-specific selective pressures and was not a direct duplicate of the microbial community in the environment. In the present study, the dominant bacterial phyla in the five carp species differed somewhat from those reported in previous studies. Fusobacteria was previously reported to be the most dominant phylum in cultured grass carp, crucian carp, and bighead carp (Li et al. 2015) and in common carp (Yu et al. 2021). Intestinal microbiota structure in perch (Perca fluviatilis) was obviously impacted by food rationing and predator presence, as Fusobacteria abundances increased under low food rations and predation stress (Zha et al. 2018). In the present study, Proteobacteria was the most dominant phylum, as was reported in previous studies of wild silver carp, bighead carp, grass carp, and common carp (Li et al. 2018). We recognized that variations of intestinal bacterial communities exist between species and individuals, so we used a large sample size from a rice-fish system with the long-term irrigation using aquaculture wastewater and focused on comparing soil bacterial communities and intestinal bacterial communities in each of five co-cultured fish species.

The co-culture of carp species
The co-culture of carp species is common in some Asian regions. Based on the mentioned reports, we recognized that aquaculture wastewater impacts the soil bacterial communities. However, it is relatively difficult to compare the impacts of fish species in the co-culture model, in comparison to the mono-culturing these fish species and irrigating rice field separately. The aquaculture operations in the co-culture and mono-culture modes were different, and the co-culture of these species optimized the use of feed and space, lowered the cost and increased the production. Under the co-culture model, phytoplankton is usually cultivated in aquaculture ponds to lower the pollutant level, particularly ammonia which is well known to have the severe biological toxicity to aquatic animals. Phytoplankton breeds zooplankton. Silver carp and bighead carp are routinely cultured to control the phytoplankton and zooplankton levels in these aquaculture ponds. Other carp species usually live on the artificial compound feeds. In this study, our results showed
that soil bacterial communities were more similar to that in common carp and grass carp intestines than the other three carp species, on the whole or on most of dominant bacterial phyla.

In carp aquaculture, the co-cultured species and their proportions differ among different regions and even within farms depending on culture models and market requirements. In the region where the present study was conducted, grass carp and crucian carp are the main culture species, but common carp are also popular with local consumers. Silver carp and bighead carp are popular in many regions of south and east China, but they play minor roles in the co-culture ponds of northwest China. The co-culture model used in the farm evaluated in this study is common in northwest China. In this study, the intestinal and soil bacterial communities were obviously different. Many factors impact the soil bacterial communities, and aquaculture operations impact environments in many ways, such as fish mucus (Molina and Fernandez 2020). Fish feces are merely one factor. The impacts of intestinal bacteria on soil bacterial communities under different co-cultured models remain yet unclear. In this system, the similarity between intestinal and soil bacterial communities was high for common carp and relatively low for crucian carp, indicating that intestinal bacteria in the numerically dominant fish species were not always more similar to the rice field soil bacteria than those of less abundant carp species.

**Source tracking**

*Cyanobacteria* represent one of the earliest branches of biological evolution on Earth, and they have been subjected to various selective pressures over time (Esteves-Ferreira et al. 2018). The interactions between *Cyanobacteria* and plants have occurred in different ways and at different levels and have been both beneficial or harmful. In recent years, interest in *Cyanobacteria*-plant interactions has grown, especially in rice-growing areas where the most efficient nitrogen-fixing *Cyanobacteria* are present (Bahareh et al. 2021). In this study, no significant differences in *Cyanobacteria* abundances were found between intestinal and soil bacteria. Members of another phylum, *Chloroflexi*, were found to be dominant in rice field soil, especially at the mature stage (Sohn et al. 2016), and in soil from rice-cray co-culture fields (Jiang et al. 2021). In our study, *Chloroflexi* was the top phylum in rice field soil, and the relative abundances were significantly higher in the soil than in fish intestines. Furthermore, the community structures of most analyzed soil bacterial phyla were more similar to that in common carp and grass carp, but soil *Firmicutes* communities were more similar to that in crucian carp. These results suggest that certain intestinal bacterial phyla in one fish species may be more similar to that in soil than other bacterial phyla.

However, there is a distance between the bacterial community similarity and the potential impacts. SourceTracker is a Bayesian approach which can be used to estimate the proportion of contaminants and possible source environments (Knights et al. 2011) and proved highly effective at predicting the composition of known sources (Brown et al. 2019; McGhee et al. 2020). Using the SourceTracker approach, Zhou et al. (2021) found that the sediment was a more important source of bacteria to the shrimp gut than the pond water and Sun et al. (2021) found that manure and original soil were the main source of the microbiome and resistome of the surface soil and rhizosphere soil. Our SourceTracker result revealed that a low contribution of intestinal bacterial communities to soil although the high proportion of shared bacterial genera, indicating intestinal bacteria were not the main sources of soil bacterial communities. In addition, the result also showed that bighead carp and crucian carp showed a relatively higher effect on soil bacterial communities than other carp species, indicating that the numerically dominant carp species do not necessarily impact soil more significantly. The analysis results on certain intestinal bacterial phyla also showed that one fish species may impact soil bacterial communities more significantly in some bacterial taxa than others.

In conclusion, although a high proportion of bacterial genera shared by intestinal and soil bacterial communities, the results from analysis of similarity and SourceTracker analysis showed that a low similarity and contribution of intestinal bacterial communities in co-cultured carps to soil communities in rice fields irrigated with aquaculture wastewater. Our comparison of intestinal bacterial communities in each carp species and soil bacterial communities also indicated that the dominant fish species in the co-cultured system did not necessarily have a more significant impact on the soil bacterial communities than less abundant fish species. Moreover, intestinal bacterial communities in a single fish species impacted certain soil bacterial phyla more significantly than others.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s13568-022-01475-x.

**Additional file 1:** Figure S1. Location of sampling sites.

**Additional file 2:** Figure S2. Community heatmap showing the top 50 genera.

**Additional file 3:** Figure S3. Venn diagrams showing the number of shared bacterial genera by soil and intestinal bacterial communities in each carp species.
Additional file 4: Table S1. Sample information and alpha diversity indexes.
Additional file 5: Table S2. The OTU table.

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Author contributions
KJL and WG supported the research and completed the design. WG and KL performed the field investigation and sample collection. KJL wrote the manuscript. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data that support the findings of this study are available in the supplementary information of this article. The raw gene sequences obtained in this study have been submitted to the NCBI SRA database (https://submit.ncbi.nlm.nih.gov/sra/) under the accession number PRJNA741343.

Declarations

Ethics approval and consent to participate
All experiments involving animals were performed in accordance with protocols approved by the Animal Ethics Committee of Shanghai Ocean University (Approval ID: SHOU-DW-2020-057).

Consent for publication
The authors agree to the journal to publish.

Competing interests
The authors declare that they have no conflicts of interest.

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References
Bahareh N, Bouaïcha N, Metcalf JS, Pozzani SJ, Konur O (2021) Plant-cyanobacterial interactions: Beneficial and harmful effects of cyanobacterial bioactive compounds on soil-plant systems and subsequent risk to animal and human health. Phytochemistry 192:112959
Balidian P (2019) The known and the unknown in soil microbial ecology. FEMS Microbiol Ecol. https://doi.org/10.1093/femsec/fiz005
Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Baldrian P (2019) The known and the unknown in soil microbial ecology. FEMS Microbiol Ecol. https://doi.org/10.1093/femsec/fiz005
Brown CM, Mathai PP, Loesekarn T, Staley C, Sadowsky MJ (2019) Influence of library composition on SourceTracker predictions for community-based microbial source tracking. Environ Sci Technol 53:60–68
Cabello FC, Godfrey HP, Ivanova L, Shah SQA, Sorum H, Tomova A (2020) Freshwater salmon aquaculture in Chile and transferable antimicrobial resistance. Environ Microbiol 22:559–563
Chang BV, Chang YT, Chao WL, Yeh SL, Kuo DL, Yang CW (2019) Effects of sulfamethoxazole and sulfamethoxazole-degrading bacteria on water quality and microbial communities in milkfish ponds. Environ Pollut 252:305–316
Chen L, Feng Q, Li C, Wei Y, Zhao Y, Feng Y, Zheng H, Li F, Li H (2017) Impacts of acupuncture wastewater irrigation on soil microbial functional diversity and community structure in and regions. Sci Rep 7:11193
Chen S, Zhou Y, Chen Y, Gu J (2018) fastp: an ultra-fast all-in-one FASTQ preprocessing tool. Bioinformatics 34:884–890
Choi S, Sim W, Jiang D, Yoon Y, Ryu J, Oh J, Woo JS, Kim YM, Lee Y (2020) Antibiotics in coastal aquaculture waters: Occurrence and elimination efficiency in oxidative water treatment processes. J Hazard Mater 396:122585
De Schryver P, Vadstein O (2014) Ecological theory as a foundation to control pathogenic invasion in aquaculture. ISME J 8:2360–2368
Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996–998
Esteves-Ferreira AA, Inaba M, Fort A, Araujo WL, Sulpice R (2018) Nitrogen metabolism in cyanobacteria: metabolic and molecular control, growth consequences and biotechnological applications. Crit Rev Microbiol 44:541–560
Garbeva P, van Veen JA, van Elsas JD (2004) Microbial diversity in soil: selection microbial populations by plant and soil type and implications for disease suppressiveness. Annu Rev Phytopathol 42:243–270
Grabicova K, Grabic R, Fedorova G, Vojs Stanova A, Blaha M, Randak T, Brooks BW, Zlavek V (2020) Water reuse and aquaculture: pharmaceutical bioaccumulation by fish during tertiary treatment in a wastewater stabilization pond. Environ Pollut 267:115593
Han GF, Zhao S, Zhang XR, Wang XL, Song C, Wang SG (2020) Distribution, combined pollution and risk assessment of antibiotics in typical marine aquaculture farms surrounding the Yellow Sea, North China. Environ Int 138:105551
Hermans SM, Breedon H, De Rycke P, Curran-Courrane F, Taylor M, Lear G (2020) Using soil bacterial communities to predict physico-chemical variables and soil quality. Microbiome 8:1–13
Huang M, Tian A, Chen J, Cao F, Chen Y, Li L (2020) Soil bacterial communities in three rice-based cropping systems differing in productivity. Sci Rep 10:9867
Jiang X, Ma H, Zhao QL, Yang J, Xin CY, Chen B (2021) Bacterial communities in paddy soil and ditch sediment under rice-crab co-culture system. AMB Express 11:163
Kim K, Hur JW, Kim S, Jung JY, Han HS (2020) Biological wastewater treatment: comparison of heterotrophs (BFT) with autotrophs (ABFT) in aquatic systems. Bioresour Technol 296:122293
Knight R, Kelley ST (2011) Bayesian community-wide culture-independent microbial source tracking. Nat Methods 8:761–763
Kumar S, Suyal DC, Yadav A, Shouche Y, Goyal R (2019) Microbial diversity and soil physicochemical characteristic of higher altitude. PLoS ONE 14:e0213844
Li T, Long M, Gousetou FJ, Zhang Q, Li A, Gong X (2015) Comparative analysis of the intestinal bacterial communities in different species of carp by pyrosequencing. Microb Ecol 69:25–36
Li X, Xu LY, Li C, Yan Q (2018) Comparative study on the gut microbiota of four economically important Asian carp species. Sci China Life Sci 61:696–705
Li D, Prinyawiwatkul W, Tan Y, Luo Y, Hong H (2021a) Asian carp: A threat to American lakes, a feast on Chinese tables. Compr Rev Food Sci Food Saf 20:2968–2990
Li Y, Li L, Yu Y, Hu Q, Li X (2021b) Impact of dietary protein content on soil bacterial and fungal communities in a rice-cob co-culture system. Front Microbiol 12:696427
Li K, Guan W, He P, Li KJ (2022) Comparison of bacterial communities on the surface of concrete breakwater structures and ambient bacterioplankton. Lett Appl Microbiol. https://doi.org/10.1111/lam.15787
Li K, Zhao S, Guan W, Li KJ (2022b) Planktonic bacteria in white shrimp (Litopenaeus vannamei) and channel catfish (Ictalurus punctatus) aquaculture ponds in a salt-alkaline region. Lett Appl Microbiol 74:212–219
Liu K, Hou J, Liu Y, Hu A, Wang M, Wang F, Chen Y, Gu Z (2019) Biogeography of the free-living and particle-attached bacteria in Tibetan lakes. FEMS Microbiol Ecol. https://doi.org/10.1093/femsec/fiz088
Magoč T, Salzberg SL (2011) FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963
Martins P, Cleary DF, Pires AC, Rodrigues AM, Quintino V, Calado R, Gomes NC (2013) Molecular analysis of bacterial communities and detection of potential pathogens in a recirculating aquaculture system for Scophthalmus maximus and Solea senegalensis. PLoS ONE 8:e60847
McGhee JJ, Rawson N, Bailey BA, Fernandez-Guerra A, Sisk-Hackworth L, Kelley ST (2020) Meta-SourceTracker: application of Bayesian source tracking to shotgun metagenomics. PeerJ 8:e8783
Molina V, Fernandez C (2020) Bacterioplankton response to nitrogen and dissolved organic matter produced from salmon mucus. Microbiologynopen 9:e1132
Nakajima T, Hudson MJ, Uchiyama J, Makibayashi K, Zhang J (2019) Common carp aquaculture in Neolithic China dates back 8000 years. Nat Ecol Evol 3:1415–1418
Neissi A, Paffie G, Farahmand H, Rahimi S, Mijakovic I (2020) Cold-resistant heterotrophic ammonium and nitrite-removing bacteria improve aquaculture conditions of rainbow trout (Oncorhynchus mykiss). Microb Ecol. 80:266–277
Ni M, Liu M, Lou J, Mi G, Yuan J, Gu Z (2021) Stocking density alters growth performance, serum biochemistry, digestive enzymes, immune response, and muscle quality of largemouth bass (Micropterus salmoides) in in-pond raceway system. Fish Physiol Biochem 47:1243–1255
Phelps QE, Tripp SJ, Bales KR, James D, Habib RA, Herzog DP (2017) Incorporating basic and applied approaches to evaluate the effects of invasive Asian Carp on native fishes: a necessary first step for integrated pest management. PLoS ONE 12:e0184081
Qi Z, Shi R, Yu Z, Han T, Li C, Xu S, Xu S, Liang Q, Yu W, Lin H, Huang H (2019) Nutrient release from fish cage aquaculture and mitigation strategies in Daya Bay, southern China. Mar Pollut Bull 146:399–407
Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590-596
Rungwangwa E, Verdegem M (2015) Microorganisms in recirculating aquaculture systems and their management. Rev Aquac 7:e37818
Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BL, Parks DH, Robinson CJ, Sahl AJW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbial 75:1601–1605
Sohn SJ, Oh YJ, Kim BY, Cho HS (2016) Effects of CaMSR2-expressing transgenic rice cultivation on soil microbial communities. J Microbiol Biotechn 26:1303–1310
Srinivasan S, Hoffm NG, Morgan MT, Matsen FA, Fiedler TL, Hall RW, Ross FJ, McCoy CO, Bumgarner R, Marranzo JM, Fredricks DN (2012) Bacterial communities in women with bacterial vaginosis: high resolution phylogenetic analyses reveal relationships of microbiota to clinical criteria. PLoS ONE 7:e30440
Sun F, Wang C, Chen L, Wang G, Zheng Z (2020) The intestinal bacterial community of healthy and diseased animals and its association with the aquaculture environment. Appl Microbiol Biotechnol 104:775–783
Sun Y, Snow D, Wala H, Li X (2021) Transmission routes of the microbiome and resistome from manure to soil and lettuce. Environ Sci Technol 55:11102–11112
Ta AT, Babel S (2020) Microplastics pollution with heavy metals in the aquaculture zone of the Chao Phraya River Estuary, Thailand. Mar Pollut Bull 161:111747
Tartaglia M, Bastida F, Scirrall R, Guarino C (2020) Soil metaproteomics for the study of the relationships between microorganisms and plants: a review of extraction protocols and ecological insights. Int J Mol Sci 21:8455
Van Tung T, Tran QB, Phuong Thao NT, Li Q, Hieu TT, Le S, Tuan NQ, Sonne C, Lam SS, Hai LT, Le QV (2021) Recycling of aquaculture wastewater and sediment for sustainable corn and water spinach production. Chemosphere 268:129529
Wang Q, Gamy GT, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of RNA sequences into the new bacterial taxonomy. Appl Environ Microbial 73:5261–5267
Wang Q, Li Z, Lian Y, Du X, Zhang S, Yuan J, Liu J, De Silva SS (2016) Farming system transformation yields significant reduction in nutrient loading: Case study of Hongze Lake, Yangtze River Basin, China. Aquaculture 457:109–117
Wu S, Wang G, Angert ER, Wang W, Li W, Zou H (2012) Composition, diversity, and origin of the bacterial community in grass carp intestine. PLoS ONE 7:e30440
Xiao Z, Zhu Y, Gao C, Zhang Y, Gao Y, Zhao Y (2022) Microbial inoculations improved rice yields by altering the presence of soil rare bacteria. Microbiol Res 254:126910
Yang Z, Xu G, Ge X, Liu B, Xu P, Song C, Zhou Q, Zhang H, Shan F, Sun C (2019) The effects of crowding stress on the growth, physiological response, and gene expression of the Nr2-Keap1 signaling pathway in blunt snout bream (Megalobrama amblycephala) reared under in-pond raceway conditions. Comp Biochem Physiol A Mol Integr Physiol 231:19–29
Yu H, Zhang Y, Zhang P, Shang X, Lu Y, Fu Y, Li Y (2021) Effects of fluoxetine on intestinal structural integrity and microbiota composition of common carp. Biol Trace Elem Res 199:3489–3496
Zadinelov IV, Dos Santos LD, Cagol L, de Muniz GB, de Souza Neves Ellendersen L, Alves HJ, Bombardelli RA, (2018) Adsorption of aquaculture pollutants using a sustainable biopolymer. Environ Sci Pollut Res Int 25:4361–4370
Zha Y, Eiler A, Johansson F, Svanback R (2018) Effects of predation stress and food ration on perch gut microbiota. Microbiome 6:28
Zhang W, Beltz B, Edwards P, Henriksson PAG, Little DC, Newton R, Treem M (2022) Aquaculture will continue to depend more on land than sea. Nature 603:2–4
Zhao D, Xu H, Zeng J, Cao X, Huang R, Shen F, Yu Z (2017) Community composition and assembly processes of the free-living and particle-attached bacteria in Taihu Lake. FEMS Microbiol Ecol. https://doi.org/10.1093/femsec/fiz062
Zhao Z, Chu C, Zhou D, Wang Q, Wu S, Zheng X, Song K, Lv W (2021) Soil bacterial community composition in rice-fish integrated farming systems with different planting years. Sci Rep 11:10855
Zhou L, Qu YY, Qin JG, Chen LQ, Han FL, Li ER (2021) Deep insight into bacterial community characterization and relationship in the pond water, sediment and the gut of shrimp (Peneaus japonicus). Aquaculture 539:736658

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