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Changes in microbial community structure and co-metabolism during the domestication of ofloxacin-degrading bacteria

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Abstract

Background: Ofloxacin (OFL) is stable and difficult to degrade. It has been detected in water, soil, and plants throughout the world. This study domesticated OFL-contaminated livestock manure soil with simplified carbon sources to identify flora capable of effectively degrading OFL. The changes in the structural composition and diversity of the microbial community and the functional abundance of the soil flora were analyzed by metagenome sequencing technology. The Biolog-ECO microplate method was used to study the utilization of 31 different carbon sources by selected bacteria and to identify the best co-metabolized carbon source for degradation.

Results: Amino acid carbon sources were more likely to cause significant changes in community structures with increasing OFL concentrations during the acclimation stage. The abundance of *Sphingobacterium* decreased from 69.23% to 9.84%, while *Alcaligenes* increased from 0.27% to 62.79%, and *Stenotrophomonas* increased from 11.63% to 33.33%, becoming the dominant genus. The results suggested that *Stenotrophomonas* and *Alcaligenes* were potential candidate bacteria for the degradation of quinolone antibiotics, such as OFL. Compared with the first stage of acclimation, there was an 87% increase (the concentration was 30 mg·L^{-1}) in the OFL degradation rate by functional flora obtained by gradient acclimation, and the functional abundance of the microbial community also increased and stabilized with the depth of the domestication process. The most significant changes in membrane transport were observed in the functional abundance of the microbial community, and it was found that itaconic acid, Tween 80, and L-aspartic acid could increase the biomass of the microbial community under OFL stress.

Conclusion: Significant changes in the bacterial composition and functional abundance of the microbial community resulted from the addition of amino acid carbon sources, together with the OFL concentration. Functional flora resulting from domestication were better able to degrade OFL. The addition of a co-metabolic carbon source significantly enhanced the biomass of the functional flora. In this study, co-metabolism was performed by adding specific carbon sources, thus achieving metabolic diversity of functional flora and ultimately efficient biodegradation of OFL. This was an important discovery in the field of microbial remediation of environmental contamination.

Keywords: Ofloxacin, Microbial community, Biostimulation, Co-metabolism

Background

Fluoroquinolones (FQs) are internationally recognized as essential broad-spectrum antibacterial drugs [1]. Due to their superior pharmacokinetic properties and ease of synthesis, these drugs are widely used in animal husbandry and the medical industry. However, they also cause serious environmental pollution [2]. Data show that from 2005 to 2016, significant amounts of 12 antibiotics

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were found in the water and sediment samples of seven major rivers and four sea areas; these antibiotics included all of the FQs [3]. Li and colleagues [4] investigated nine sampling points in the Beijing–Tianjin–Hebei region and extracted wastewater and groundwater from pig farms, as well as groundwater from nearby villages for measurement. High concentrations, reaching mg·L^{-1}, of FQs were found, especially, in the wastewater from pig farms. In the past decade, the environmental concentrations of FQs have increased rapidly and the scope of influence has expanded. Zhang and colleagues [5] studied the temporal and spatial variation of FQs in water and sediment samples from Baiyangdian finding that, during the period from 2013 to 2019, the concentrations of FQs in this area had increased at least tenfold, with concentrations ranging from 0.738 to 3093 ng·L^{-1}. Among them, flumequine and ofloxacin were detected with the highest frequency. Mo Yuanmin and associates [6] found that the concentrations of ofloxacin, norfloxacin, ciprofloxacin, and enrofloxacin in Qingshitan reservoir water were 50.00–660.13, 3.70–5.00, 3.49–6.22, and 4.59–6.06 ng·L^{-1}, respectively, with FQs not only increasing rapidly in concentration but also in the scope of influence over the previous decade of environmental monitoring. FQs have also been detected in wastewater, surface water, and sediments in most regions of Africa [7]. In wastewater, FQs are mainly found as deposits in sludge through adsorption and the bioaccumulation of their residues leads to environmental risks in terms of land use and resource utilization [8]. Liu Feng and colleagues [9] investigated antibiotic accumulation in livestock and poultry manure, manure, crop soil, ditch bottom mud, and constructed wetland soil in livestock and poultry farms in Xiamen and Putian, Fujian, and found that quinolone antibiotics (QNs) were the most abundant, with a total content of a single sample reaching up to 2967.6 μg·kg^{-1} in manure and 579.0 μg·kg-1 in crop soil. It has also been suggested that it was reported that due to their high chemical stability, FQs not only persist in the environment for extended periods but also promote the growth of antibiotic-resistant bacterial colonies through the selection of antibiotic resistance genes in China [10]. As bacterial resistance increases, the FQs spread more widely and become more difficult to degrade biochemically [11]. FQs not only affect the dynamics of biological populations but also enter the human body through the food chain, posing a major threat to human health [12–14]. Therefore, the removal of residual antibiotics in the environment has become a major concern and research hotspot.

Despite some limitations, biodegradation remains one of the most cost-effective methods for the removal of antibiotics and their residues from the environment [15, 16] as microorganisms can either degrade the antibiotics directly or indirectly through the use of their own metabolic secretion products (such as extracellular polymers and enzymes) to catalyze decomposition. Traditional microbial degradation uses pollutants as carbon and energy sources for the growth and metabolism of microorganisms. Although biodegradation of some FQs is difficult, many are able to be transformed or degraded by microorganisms in the presence of external carbon sources, described as the co-metabolism of microorganisms and external carbon sources [17]. Co-metabolism can be stimulated by the addition of nutrients that are easily degraded by microorganisms, such as amino acid carbon sources, which can provide carbon sources and energy for microorganisms, maintain cell metabolic activity, or promote the activity of microbial enzymes responsible for degradation. These methods both promote microbial growth and enhance the degradation of pollutants [18, 19]. Therefore, research into antibiotic degradation will provide the necessary theoretical basis for the practical application of microbial environmental remediation in the future. Despite increasing evidence of the ecological implications of FQ contamination, there are relatively few reports on the potential applications of adding co-metabolic carbon sources to enhance microbial degradation of FQs. It is also unclear how and what effects carbon sources and FQs have on the composition of the primitive flora of the soil during the co-metabolism of different carbon sources. In this study, ofloxacin (OFL), a typical FQ with a high detection rate in the environment, was selected as the target pollutant [5]. We used the existing environmental microbial flora to degrade OFL, finding that the addition of different metabolic carbon sources significantly improved the degradation efficiency of OFL by changing the species richness and structural composition of the flora, as well as the microbial biomass of the functional flora.

The primary objectives of this study were (1) to study the changes and differences in the compositions of the microbial community structures in OFL-contaminated soil during the three domestication processes; (2) to add different types of carbon sources as stimuli, and screen to obtain high-efficiency degradation in the natural environment. The dominant flora were analyzed by metagenomic sequencing to evaluate changes in the abundance of functional genes to investigate their potential functions in antibiotic-contaminated environments; (3) to study the utilization of 31 different carbon sources by the identified high-efficiency antibiotic-degrading bacteria. The optimal co-metabolic carbon source for bacterial flora degradation was identified, and it is hoped that the co-metabolic principle will be explored in the future to provide strong theoretical support for the practical

application of functional microorganisms in the remediation of antibiotic-contaminated environments.

Materials and methods

Main reagents

Soil samples were collected from the area around a livestock and poultry farm in Yanshan town, Guilin, Guangxi. 5 locations were selected as target sampling sites, and two types of soil (surface soil and soil 10 cm below ground level) were collected, and the sampling method was random. The different types of soil were mixed into 2 parts and brought back in sterile self-sealing bags and placed in a refrigerator at 4 °C. One part was subjected to colony domestication experiments within 24 h, and another sample was taken for physicochemical property analysis. The media used were: (1) Inorganic salt medium: K_2HPO_4 5.8 g·L^{-1}; KH_2PO_4 4.5 g·L^{-1}; $(NH_4)_2SO_4$ 2.0 g·L^{-1}; $MgCl_2$ 0.16 g·L^{-1}; $CaCl_2$ 0.02 g·L^{-1}, adjusted to pH 7.0. (2) Basic medium: tryptone 10.0 g·L^{-1}; yeast extract 5.0 g·L^{-1}; NaCl 10.0 g·L^{-1}, pH 7.5. The medium was sterilized at 121 °C for 30 min before use. (3) Phosphate buffer: NaCl 8 gL^{-1}; KCl 0.2 g·L^{-1}; KH_2PO_4 0.2 g·L^{-1}, pH 7.0. (4) OFL standard stock solution: 1.0 g OFL was added into 100 mL deionized water and stirred using a magnetic stirrer in the dark until completely dissolved. The OFL stock solution was stored shielded from light at 4 °C. (5) The amino acid mixture was L-arginine hydrochloride, L-cystine, L-isoleucine, leucine, L-histidine hydrochloride–hydrate, L-lysine salt acid, L-methionine, L-phenylalanine, L-threonine, L-tryptophan, L-tyrosine, and L-valine, with a final concentration of 10 mg·L^{-1}. The vitamin mixture was choline chloride, calcium D-pantothenate, folic acid, niacinamide, pyridoxal hydrochloride, riboflavin, thiamine hydrochloride, and i-inositol, all at a concentration of 1 mg·L^{-1}. The domestication of OFL-degrading bacteria was performed using the methods currently in use in our laboratory, as previously described [20]. Enriched bacteria (ECI, ECII, ECIII) were obtained after domestication. The colonies obtained in the first stage of domestication were recorded as ECI, the domestication method was the same as in the first stage, and the enriched bacteria obtained were designated ECII. The procedure was repeated in the third stage with an amino acid mixture as carbon source, with the other conditions remaining unchanged, to obtain ECIII(The specific domestication method was described in the Additional file 1).

Analysis method

Determination of biodegradability

Soil samples at different depths were freeze-dried for 24 h and analyzed by an elemental analyzer combined with a microbalance in parts per million, and the results are presented in the Additional file 1: Table S1. The residual concentrations of OFL in the media at different domestication stages were determined at regular intervals by HPLC and the degradation rates were calculated to screen for bacteria that degrade OFL with high efficiency [21]. At the same time, a control experiment was set up to exclude the interference of spontaneous degradation of OFL (the same operation as the acclimation process but without the addition of bacteria). The remaining OFL concentration in the system was measured every 15 days (after one domestication cycle). Fifteen milliliters of the culture solution were accurately measured, filtered through a 0.45-μm glass fiber membrane, adjusted to pH 3.0 with 4 mol·L^{-1} sulfuric acid, and then passed through the HLB solid-phase extraction cartridge at a flow rate of 5 mL·min^{-1}. The HLB solid-phase extraction cartridge was activated with 6 mL of methanol and 6 mL of ultrapure water. After the water sample had passed through the column, the cartridge was rinsed with 6 mL of ultrapure water and 6 mL of 5% methanol in aqueous solution, after which the solid-phase extraction cartridge was pumped under vacuum for 30 min to dryness, eluted with 6 mL of methanol (under natural gravity flow), and blown with nitrogen to near-dryness. The volume was diluted to 1 mL with methanol and the solution was passed through a 0.22-μm filter membrane into a 2 mL brown injection bottle and stored at − 20 °C until testing. The test conditions for high-performance liquid chromatography were: WaterXBridgeC18 column (4.6 × 150 mm, 5 μm); column temperature 25 °C; mobile phase A: acetonitrile, mobile phase B: 0.2% acetic acid aqueous solution, and A: B = 15: 85; the flow rate was 1 mL·min^{-1} with an injection volume of 20 μL; the wavelength of the UV detector was 288 nm. At the same time, OFL standard solutions with concentrations of 0 ~ 2 and 0 ~ 50 mg·L^{-1} were prepared, resulting in a linear standard curve ($R^2 \geq 0.999$).

DNA extraction

A total of six samples (divided into two groups with three samples per group) were extracted. These were liquid bacterial samples from the three stages of domestication in the surface layer and deep soil and were termed S-ECI, S-ECII, S-ECIII, Q-ECI, Q-ECII, Q-ECIII (S and Q represented different depths, S was the deep layer, and Q was the surface layer). Genomic DNA was extracted from each group using the omega E.Z.N.ATM Mag-Bind Soil DNA Kit from the bacteria from each acclimation stage.

High-throughput amplicon sequencing

The total DNA of the six samples extracted from domestication was used as a template for PCR amplification. The primers used for PCR were fused with the V3–V4 universal primers of the Illumina Miseq sequencing platform.

The sample sequences were distinguished by barcode, and after PCR and electrophoresis detection, they were sequenced on the Illumina MiSeq platform. The bacterial sequences in the sample were sequenced on the platform. The primer sequence was 341F (CCCTACACGACGCTC TTCCGATCTG (barcode) CCTACGGGNGGCWGC AG) 805R (GACTGGAGTTCCTTGGCACCCGAG AATTCCAGACTACHVGGGTATCC).The sequencing process was: sample DNA extraction; fragmentation; addition of primers at both ends of the sequence; PCR amplification and product purification. This was followed by PCR product quantification and normalization, PE library construction, onboard sequencing, and data analysis. The 16S rRNA gene sequences were demultiplexed, quality filtered, de-duplicated, sorted for abundance, OTU clustered and chimera identified using the UPARSE pipeline. Briefly, after removal of barcodes and primer sequences, reads were truncated to a length of 200 bp and only reads with quality score Q > 20 and no ambiguous bases were retained for analysis. Non-amplified region sequences were removed from the pretreated sequences using Usearch, and the sequences were then sequenced for error correction and uchime was called to identify chimeras. Subsequently, we then compared the chimera-removed sequences with representative database sequences for blastness. In addition, OTUs were defined with 97% sequence identity. The RDP classifier trained on Greengenes reference database (http://rdp.cme.msu.edu/misc/resources.jsp) was used based on Bergey's taxonomy, and the Naïve Bayesian assignment algorithm to classify OTU representative sequences. Experimental details are given in the Additional file 1.

To study the phylogenetic relationships of the different OTUs, all sample sequences were clustered according to the sequence distance, and the representative sequences of OTUs were taxonomically analyzed by the Yebes algorithm and composition. To describe the biodiversity between different samples, the Shannon index (reflecting the evenness and diversity of the community), the Chao1 index (reflecting the community richness), and the construction of a community functional abundance grading graph were used for diversity analysis [22]. Cluster analysis between samples was carried out according to species relationships, and the similarities and differences in community compositions between samples at each taxonomic level were analyzed. The composition of the functional classifications was analyzed using metagenomic sequencing, and the gene functions were examined. The functional genes of the flora were compared with the functional gene database (FGR: RDP, a functional gene database compiled from GenBank, http://fungene.cme.msu.edu/). PICRUSt was used for the prediction of metabolic function. Existing 16S rRNA gene sequencing data were mapped against microbial reference genomic databases, where metabolic function is known, thus enabling prediction of metabolic function in bacteria and archaea, as detailed in the Additional file 1.

Stimulation of bacterial growth by different carbon sources

The functional flora obtained by domestication were inoculated into the basal medium and incubated at a constant temperature with shaking (30 °C, 150 rpm) for 24 h. The bacteria were precipitated by centrifugation, rinsed three times with PBS, and diluted to a suspension of OD600 = 1.0. Bacteria were added to the inorganic salt medium, and OFL was added to final concentrations of 0, 1, 2, 5, 10, 20 $mg{\cdot}L^{-1}$, and the solution was finally diluted to $OD_{600} = 0.5$. Two hundred microliters of the solution were inoculated into Biolog-ECO 96-well plates, with three parallel samples for each concentration. The plates were incubated at 30 °C in the dark, with absorbances at 590 nm read every 24 h, for a total of 16 days. According to the average well color development (AWCD) [17] and the utilization of carbon sources, the optimal carbon source was selected as the biostimulatory carbon source for the functional flora for OFL degradation. The average discoloration rate was calculated by the following formula:

$$\mathrm{AWCD} = \sum (C_i - R)/n \tag{1}$$

where C_i is the optical density value of each reaction well at 590 nm, R is the optical density value of the control well of the ECO plate; n is the type of medium carbon source [23].

Statistical analysis

Data were analyzed by principal component analysis (PCA) using Origin. P values < 0.05 were considered significant.

Results and discussion

Analysis of community structure diversity in different domestication stages

The alpha diversities of all samples were calculated based on the number of sequences obtained by sequencing and the number of OTUs obtained by clustering. As shown in Fig. 1, it can be seen from the Chao1 and ACE indices (Fig. 1a) that the community richness in the S-ECII period was not much different from that in the S-ECI period, while the community richness in the S-ECIII period increased significantly. This indicated that under OFL stress conditions, the addition of yeast extract, a mixture of amino acids, vitamins, and adenine, did not appear to affect the bacterial community structures to any great extent. However, the stimulation of a single and

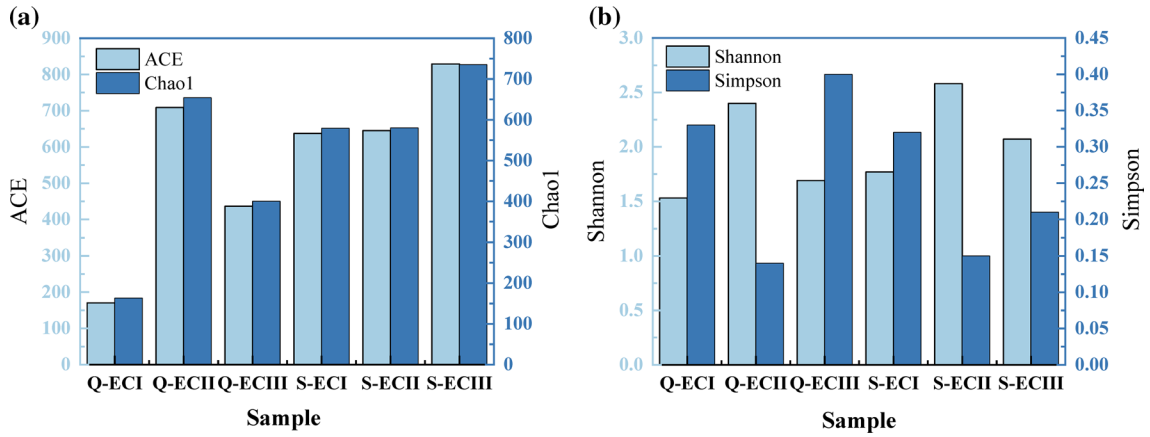

Fig.1 Graph of each correlation index of alpha diversity: **a** ACE and Chao1 index and **b** Shannon and Simpson index of samples during domestication

easily available carbon source may be beneficial to specific bacteria in the community and lead to increased biomass of genera, such as *Alcaligenes*. It can be seen from the Shannon index (Fig. 1b) that, during the three stages of domestication, the soil microbial community diversity in both groups first increased and then declined. The Simpson index calculates the probability that two OTUs are randomly selected from sample data and belong to different species. The larger the probability, the smaller the Simpson index, indicating higher species diversity in the sample [24]. In the two soil groups in this study, the Simpson index (Fig. 1b) was lowest during the second stage of domestication, indicating that the species diversity of the community was the highest after the addition of the amino acid mixture, vitamin mixture, and mixed adenine carbon source. In addition, the community structure of Q-ECI was quite different from other periods as a whole, and the microbial community structures of Q-ECII, Q-ECIII, S-ECI, S-ECII, and S-ECIII were highly similar, which was also related to the degrading bacteria. The domestication gradients of the groups corresponded to these findings. The community structure compositions tended to be similar as the domestication time increased. However, the ACE and Chao1 indices of S-ECIII and S-ECI, and S-ECII all increased, indicating that the addition of a relatively simple amino acid carbon source caused a significant change in community structure.

Venn diagrams are used for the visual presentation of the proportions of the various OTUs between samples and the similarities and overlaps between samples [25]. As shown in Fig. 2, in group S, the number of bacterial species shared between samples was relatively high (Fig. 2a). Sample S-ECIII had the most unique bacterial species, accounting for 46.5% of all bacterial species in S-ECIII. In the Q group, relatively few bacterial species were shared among the samples in the sample Q-ECI, with a total of 40 species (Fig. 2b). The unique bacterial species in Q-ECII and Q-ECIII accounted for 59.2% and 52.0% of all bacterial species, respectively. This indicated that the composition of the bacterial communities changed greatly during the different domestication processes. The OFL concentration increased during these different stages of domestication, together with continuous simplification of the types of carbon sources available to the microorganisms, allowing ease of screening and identification of bacterial communities that can efficiently degrade OFL.

PCoA analysis was used to observe and analyze the changes in microbial communities. The percentages indicate the value of the degree of explanation of the differences in sample composition by the principal axes. The three principal axes contain 93% of all the information that can show the differences between the groups of samples. Figure 3 shows the PCoA analysis at the phylum level of bacterial communities in the different domestication stages. As shown in the figure, the 6 samples were distributed in different areas, indicating significant differences in bacterial community composition between samples. PCoA1 and PCoA2 accounted for the large distance between Q-ECIII and Q-ECII, Q-ECI, indicating that the community structure of the third domestication stage had a significant change compared to the second and third stages of domestication. Similarly, S-ECIII was far from S-ECI and S-ECII, which also indicated that S-ECIII had a significant change in community structure in the third domestication stage. From the comparison between PCoA1 and PCoA3, it was found that the interval distance of ECI, ECII and ECIII samples were all far apart,

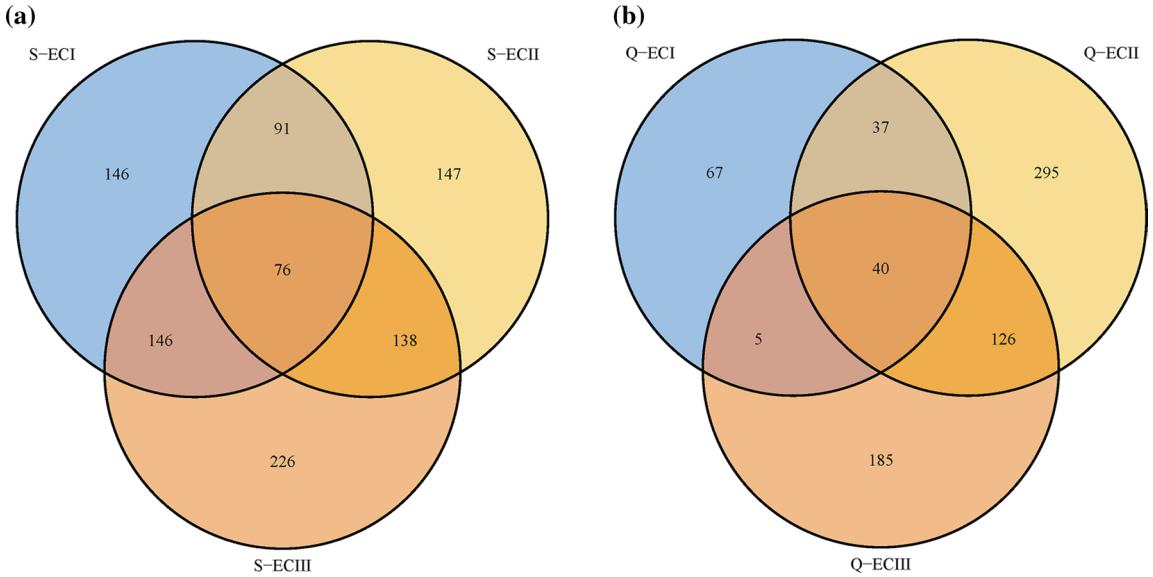

Fig.2 Venn diagram of OTU overlap between samples in **a** group S and **b** in group Q in different domestication periods

indicating that both shallow soils(Q) and deep soils(S) were more influenced by environmental carbon sources and OFL at different domestication stages, and the overall community structure changed significantly. The short interval distance between Q and S group samples and the high similarity of microbial community structure also corresponded to the domestication gradient of degrading bacteria. In the comparison of PCoA2 and PCoA3, it can be observed that the composition of S-ECIII and S-ECII community structures tended to be similar in the S group as the domestication time increased. Collectively, this indicates that there are big difference between the microbial community at each stage of domestication with the added changed carbon source. The most likely reason for these differences is that under the stress of high concentrations of ofloxacin, different bacterial groups have different absorption and utilization of different carbon sources, so there will be various community compositions and changes (see Fig. 3)

The acclimation stage of OFL-degrading bacteria was divided into three stages. The OFL concentration was increased as a gradient. This resulted in the gradual enrichment of bacteria with the ability to degrade OFL under the selection of environmental pressure. As shown in Fig. 4a, we can clearly see that during the first stage of domestication, the community composition structures of samples from different soil depths differed significantly. *Sphingobacterium chitinophaga* (69.23%),

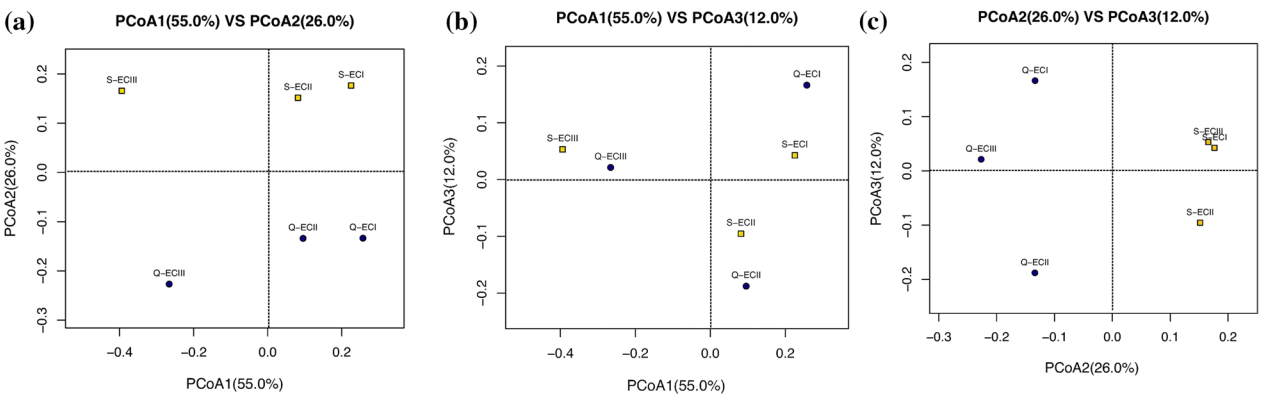

Fig. 3 PCoA analysis of bacterial community structure in the domestication stage

Stenotrophomonas (11.63%), *Escherichia coli/Shigella* (1.89%), *Paenibacillus* (1.77%), and *Bacillus* (9.72%) were present in S-ECI with over 1% relative abundance, while *Alcaligenes* (1.21%), *Sphingobacterium* (14.25%), *Escherichia coli/shigella* (37.33%), and *Bacillus* (41.9%) were found to have relative abundances of over 1% in Q-ECI. In the second stage of acclimation, the relative abundance of bacteria in the S-ECII that exceeded 1% included *Sphingobacterium chitinophaga* (34.49%), *Oligotrophic Stenotrophomonas* (22.95%), *Escherichia coli escherichia/shigella* (14.24%), *Achromobacter* (4.25%), and *Shigella* (10.72%), while *Alcaligenes* (25.69%), *Sphingobacterium* (29.84%), *Escherichia coli/Shigella* (7.68%), *Bosea* (2.9%), *Devosia* (2.94%), *Achromobacter* (15.9%), and *Hyphomicrobium* (2.55%) had relative abundances of over 1% in Q-ECII. In the third stage of acclimation, the relative abundances over 1% in S-ECIII included *Alcaligenes* (27.24%), *Sphingobacterium chitinophaga* (9.84%), *Oligotrophic Bacteria Stenotrophomonas* (33.33%), and *Achromobacter* (22.39%), while in Q-ECIII, the relative abundances over 1% were *Escherichia coli* (1.33%), *Stenotrophomonas* (7.57%), *Bosea* (4.76%), *Hyphomyces* (3.33%), *Acinetobacter* (5.15%), and *Pigmentiphaga* (3.72%). At the genus level, the proportion of microorganisms in the community structures during domestication changed significantly in response to both increased OFL concentrations and the length of domestication. Microorganisms that are able to use OFL directly as a carbon and energy source or are able to use metabolites resulting from microbial degradation as carbon sources can survive. In contrast, the proportions of those microorganisms that cannot adapt to the domesticated environmental system gradually decrease until they are eliminated. During the first stage of domestication, the community compositions of samples from different soil depths differed significantly (Fig. 4c). At each domestication stage, the OTU2 and OTU5 nodes showed significant correlations with other nodes, and their connectivity was also higher among all nodes (Fig. 4b). OTU2 and OTU5 represent *Bacteroidetes* and *Proteobacteria*, respectively. The sum of their relative abundances was high, indicating that these two types of bacteria also occupied dominant positions in the composition and evolution of microbial communities in the process of domestication, and were most likely to be potential OFL-degrading bacteria genera. As domestication lengthened and the external carbon sources became simplified, groups with poor tolerance and adaptability gradually disappeared. For example, when yeast extract was added as an external carbon source in the deep soil after domestication, there was a high abundance of *Sphingobacterium* (69.23%). However, as the carbon source type and OFL concentration continued to decrease, the relative abundance of *Sphingobacterium* decreased gradually. When additional carbon sources, such as the amino acid mixture, vitamins, and adenine, were included, the relative abundance decreased to 34.49% and when the carbon source was adjusted to include the amino acid mixture only, the relative abundance decreased to 27.74%. Thus, with gradual increases in the OFL concentration and the addition of only amino acids as the carbon source, the abundance of certain microorganisms, such as *Sphingobacterium,* could be reduced and others, such as *Alcaligenes* and *Stegotrophomonas* could assume dominance(as shown in Fig. 4c). Thus, it can be seen that amino acid carbon sources significantly impact the natural community structures of antibiotic-contaminated soils.

The sample clustering tree diagram intuitively reflects the similarities and differences between multiple samples through the branch structure [26]. These relationships can be seen more clearly using cluster analysis and the functional abundance histogram. The results (Fig. 5a) showed that microbial community structures in the deep soil samples were strongly similar in composition, with little change in functional abundance. As the domestication process progresses, the community structures became more varied, as seen in the microbial community compositions of surface soil samples, where the similarity was lower and the abundance in Q-ECI was poor; however, as domestication progresses, the community structures of Q-ECII and Q-ECIII tended to be similar. The functional abundance also increased and tended to be stable. The most significant changes were seen in the abundance of functions associated with membrane transport. This may be because the accumulation of OFL in cells is related to active transport systems in the cell membrane [27]. As the OFL concentration increases, the microbial cells increase their capacity for membrane transport leading to the accumulation of OFL within the cell. These microorganisms may then increase the secretion of polymers and enzymes to catalyze OFL decomposition of OFL, and these microorganisms may thus

(See figure on next page.)

Fig. 4 **a** Combination analysis graph of sample clustering tree and histogram based on KEGG enrichment at different domestication stages and **b** microbial correlation network diagram (Note: the node pie chart represents different OTUs, and different color blocks on the pie chart represent the abundance of samples in the OTU classification. Red lines indicate positive correlations; green lines indicate negative correlations; significant correlations ($p < 0.05$) are shown by dotted lines; highly significant correlations ($p < 0.01$) are shown by solid lines; correlations greater than 0.8 are indicated by thick lines and correlations below 0.8 are indicated by thin lines. **c** Heatmap of genus level species abundance at different domestication stages

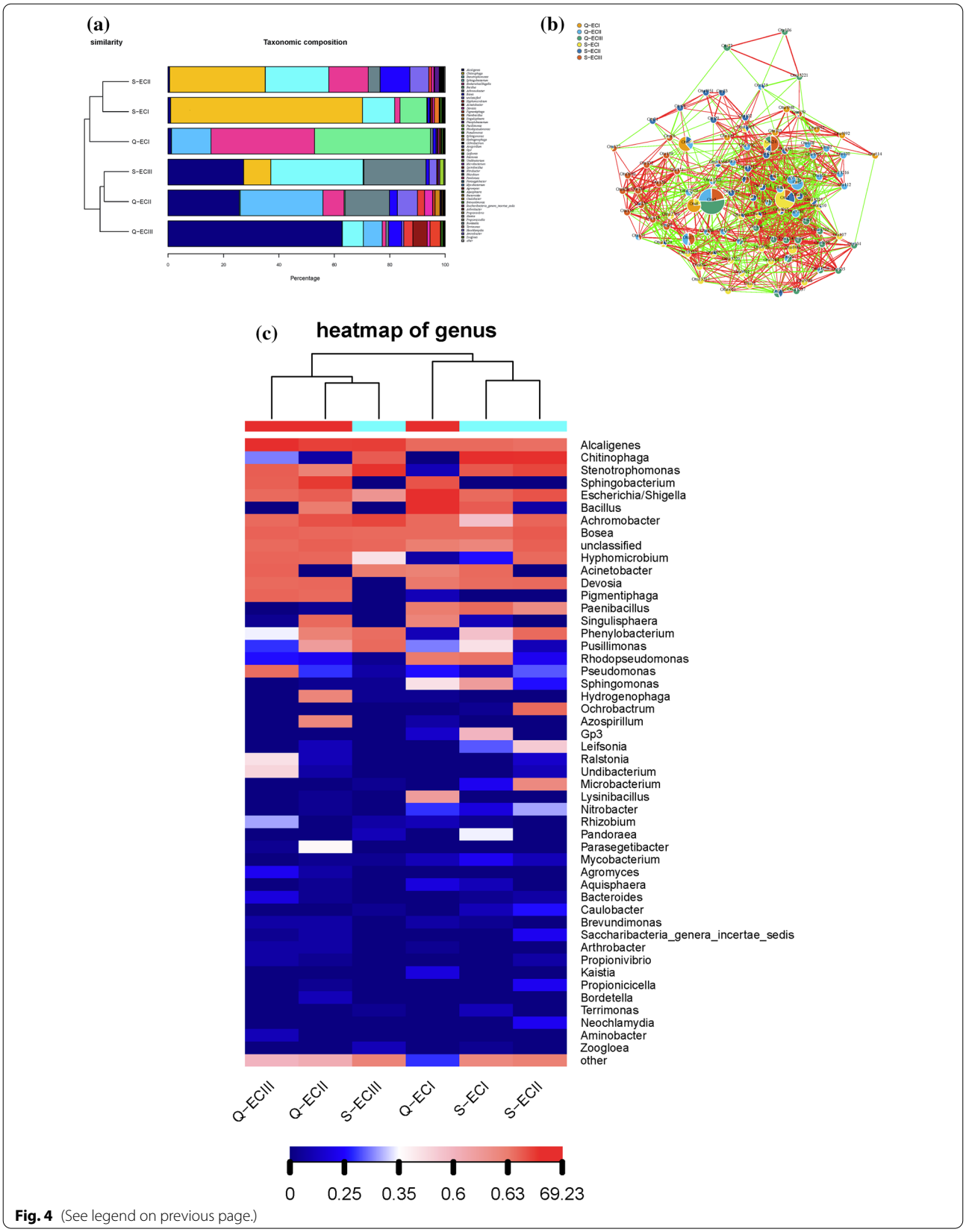

Fig. 4 (See legend on previous page.)

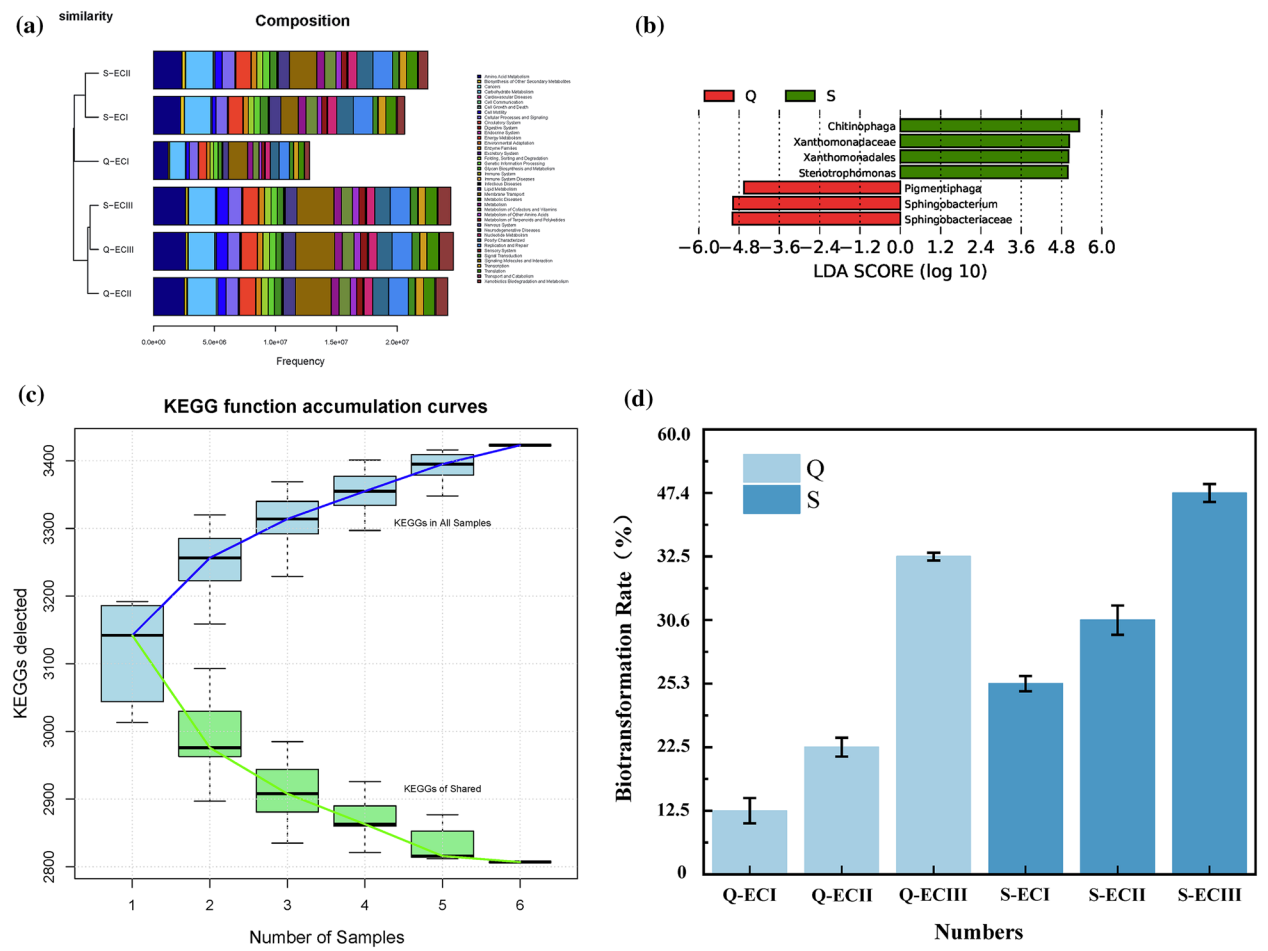

Fig.5 **a** Combination analysis of clustering tree and histogram of all samples at the genus level and **b** Lefse analysis histogram. Different colors represent different groups, the horizontal axis is the LDA score obtained after LDA analysis, and the vertical axis is the microbial group with significant effect. **c** COG function cumulative curve. The horizontal axis is the number of samples, and the vertical axis is the number of COG IDs after sampling. In the figure, blue represents the collection of all sample COG IDs, and green represents the intersection of all sample COG IDs. **d** OFL Biotransformation rate in different domestication stages

become the dominant flora during the domestication process. it was found that the bacterial composition did not vary greatly between different groups, while the distribution of functional genes did. Functional redundancy among distinct microbial species may provide a mechanism to maintain the community functionality with varied community compositions, and in this mechanism, one or more microbial taxa in the community play a significant role in responding to different environments [28–30]. LEfSe (Linear discriminant analysis Effect Size) was used to identify the genetic and functional characteristics that could best explain differences between two groups of samples under different conditions of soil biology, as well as the influence of these characteristics on the inter-group differences. LEfSe analysis allowed the identification of differential features and determination of their significance, leading to the analysis of multi-level biological markers and characteristics. It was found that in group S, *Chitinophaga*, *Stenotrophomonas*, *Xanthomonadales*, and *Xanthomonadaceae* played significant roles, while in group Q, *Pigmentiphaga*, *Sphingobacteriaceae*, and *Sphingobacterium* were found to be important (Fig. 5b); the results of the combined analysis are illustrated in the heatmap (Fig. 4c) and sample cluster tree and histogram (Fig. 4b). The results reflect the rate at which new COGs emerge under continuous sampling (Fig. 5c). A sharp rise in the curve in relation to increasing sample size within a particular range indicates the presence of many new functions in the community; in contrast, a flatter curve indicates that the number of functions in the environment have not increased significantly in relation to the sample size. The function accumulation curve can

be used to judge whether the sample size is sufficient. A sharp rise in the curve indicates that the sample size is insufficient and should be increased, while curves that rise and fall gently indicate that the sample volume is sufficient to reflect the functional abundance of the sample.

Overall, the abundance ratios of *Alcaligenes*, *Sphingobacterium*, and *Stenotrophomonas* changed the most during the domestication of deep-soil microorganisms. *Alcaligenes* increased from 0.94% to 27.24%, *Stenotrophomonas* increased from 11.63% to 33.33%, while *Sphingobacterium* decreased to 9.84%. The domestication process of surface soil microorganisms was similar, with *Alcaligenes* increasing from 1.21% to 62.79%, *Sphingobacterium* decreasing from 14.25% to 6.82%, and *Escherichia coli* decreasing from 37.33% to 1.33%. It appears that *Alcaligenes* and *Stenotrophomonas* can gradually adapt to the external domestication environment, allowing the utilization of OFL or OFL degradation products as carbon sources to carry out their life activities. It has also been shown that FQs not only greatly increase bacterial diversity and change the structure of the entire bacterial community, but also contribute to the complexity of molecular ecological networks and microbial interactions [31]. In addition, it has been shown that the microbial community structure is greatly affected by the addition of moderate amounts of yeast to the sludge, but the strongest synergistic metabolism can be established between the functional microorganisms yeast and methanogens and the best resource utilization can be achieved under these conditions [32]. Therefore, we speculate that some kind of mutualistic synergistic relationship also exists or is enhanced between Alcaligenes and Stenotrophomonas, which may improve the nutrient balance, dilute toxic compounds, improve buffering capacity, based on these synergistic effects are ultimately reflected in the microbial community. These synergistic effects are ultimately reflected in the microbial community functions [33]. Microbial correlation network diagram (Fig. 4b) further supports this notion. Indeed, the soil itself is considered a natural reservoir of antibiotic-resistant bacteria, carrying a variety of known and unknown determinants of resistance [34]. In addition, the environmental concentrations of antibiotics may act as a means of inter- and intra-domain communication and regulation in bacteria, selectively influencing the structure and diversity of bacterial communities [35, 36]. In conclusion, mixed bacterial cultures and their synergistic metabolic activities provided stability in the association between these cultures during biodegradation of OFL. Thus, the overall function of the bacterial community is not readily affected, despite the loss of some species during the domestication and biodegradation process. Combination analysis of clustering tree and histogram of all samples at the genus level (Fig. 5a) further supports this notion.

Identification of efficient OFL-degrading bacteria

The biodegradation rate data determined by HPLC are shown in Fig. 5d. From the biotransformation rate data, we can see that the deep-soil flora are better able to degrade OFL that the surface-soil flora. Of these, the biotransformation rate of OFL by S-ECIII was the highest, reaching 47.4%, and these samples were used for the selection of dominant bacteria. Unlike in the first two domestication stages, the carbon source in the third stage used only the amino acid mixture as the metabolic carbon source, and the degradation rate in S-ECI increased by 87%, indicating that the selection of the appropriate co-metabolic carbon source not only improves the species diversity of the community but also significantly enhanced the ability of the flora to degrade OFL. It was found that the biodegradation of sulfamethoxazole was also enhanced when an easily degradable energy supply (acetate) was provided which fostered metabolic activity [37]. Related studies have shown that carbon sources such as some utilization traits exhibit a significant positive correlation with the abundance of chlorane and chloroene degradation pathways [38]. This correlation can, therefore, be interpreted as promoting effective biostimulation through external nutrient amendment to increase biometabolic diversity and activity for efficient in situ degradation of contaminants. Indeed, in biological treatment, there is generally a positive correlation between the biotransformation rate of pollutants and the metabolic activity and functional diversity of the microorganisms [39–41]. In addition, based on microbial cooperation reported previously [42–44], Three types of cooperative metabolic interactions were expected to occur among the flora and culture during degradation of OFL, promoting overall metabolic activity and functional enrichment of the bacterial flora: (1) the specific nutritional deficiency of the OFL-degrading strains required for metabolism provided by secondary strains or readily decomposable carbon sources, thus promoting the degradation of OFL; (2) metabolic associations characterised by the cross-feeding of metabolites from the degradation pathway among consortium members; and(3) metabolite detoxification, including the production of toxic metabolites by OFL degrading bacteria but with environmental carbon sources providing a novel colony detoxification strategy or being consumed by secondary strains, leading to rapid degradation. As increased microbial diversity broadens the number of degradation pathways available in the reaction system, the addition of suitable exogenous substances for co-metabolism can also promote the

biotransformation of antibiotics by microorganisms [45]. As noted above the acclimated bacteria in the resulting OFL-degrading community, rather than pure cultures alone, were highly efficient for the high degradation of OFL. This was because the common metabolic processes among microorganisms also contributed to the biodegradation of antibiotics [46].

Structural analysis of the dominant flora in S-ECIII

High-throughput amplicon sequencing can comprehensively and accurately reflect the microbial community structure, allowing the acquisition of information, such as the species composition, richness, and diversity of microbial communities in specific environments [47]. At the phylum level, the OFL-degrading flora of S-ECIII belonged mainly to two phyla, namely, the Proteobacteria (88.78%) and Bacteroidetes (11.09%). At the class level, the OFL-degrading flora included Betaproteobacteria (52.48%) and Gammaproteobacteria (34.16%), while at the genus level, the main bacterial genera were *Stenotrophomonas* (33.33%), *Alcaligenes* (27.24%), and *Achromobacter* (22.39%) (Fig. 6). Several studies have found that the most common bacterial phyla in antibiotic-contaminated anaerobic digested sludge and various antibiotic-contaminated swine manure compost soils are Proteobacteria and Bacteroidetes [11, 23, 41, 48]. Bacteria can degrade various organic pollutants, such as organic pesticides, and thus have vital bioremediation functions [49]. *Alcaligenes* can remove environmental pollutants containing nitrogen elements or benzene rings. The FQ structure also includes nitrogen and benzene rings, suggesting that *Alcaligenes* have potential value for the removal of antibiotic pollutants and the restoration of environmental organisms [50]. This study also obtained consistent results through the phylum-level analysis of the dominant bacterial community structures after domestication. Therefore, it can be considered that *Alcaligenes* and *Stenotrophomonas* in Proteobacteria are potentially highly efficient OFL-degrading bacteria, playing a major role in the biotransformation of OFL.

PCA analysis of carbon source utilization by bacteria under OFL stress

Figure 7A shows the utilization of six carbon sources by functional flora under different OFL concentrations. The data analysis of the absorbance values of the six types of carbon sources on Biolog-ECO plates cultured for 16 days showed that the identified dominant bacteria had higher utilization rates of four types of carbon sources, namely, amines, amino acids, lipids, and carboxylic acids. Of these, utilization of amines was the highest. Lower concentrations of OFL (<5 mg·L^{-1}) can promote the utilization of amines by available flora. At high OFL concentrations (>5 mg·L^{-1}), the antibiotic significantly inhibited the utilization of various carbon sources by the functional bacteria. There were significant differences in the utilization rate of carbon sources such as amino acids but no substantial changes in the utilization rate of polymeric carbohydrates.

After treatment with different concentrations of OFL, it was found that the OFL concentration suitable for the growth and metabolism of functional flora was 5 mg·L^{-1} (as shown in Additional file 1: Fig. S1). Therefore, principal component analysis was performed on data points cultured for 16 days in a medium with an OFL concentration of 5 mg·L^{-1} to study the metabolic utilization of 31 carbon sources by the functional flora. As shown in Fig. 7b, the cumulative contribution rates of the two principal components (PC1 and PC2) were 70.1% and 16.6%, respectively, while the total cumulative eigenvalue contribution rate of the two principal components of the pivot vector was 86.7%. The second principal component (PC2) represents the primary source of variation. From the loading diagram of 31 carbon sources on the two principal components (Table 1), it can be seen that itaconic acid of the hydroxy acids, Tween 80 of the esters, and L-aspartic acid of the amino acids are the main carbon sources affecting PC1. The main carbon sources affecting PC2 were methyl pyruvate of the esters, Tween 40, and D-galacturonic acid of the hydroxy acids.

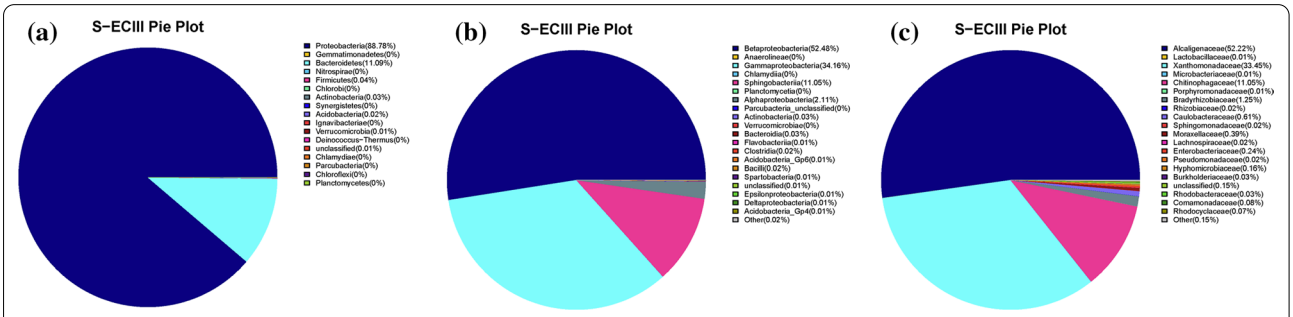

Fig. 6 S-ECIII community composition structures. **a**, phylum level; **b**, class level; **c**, genus level

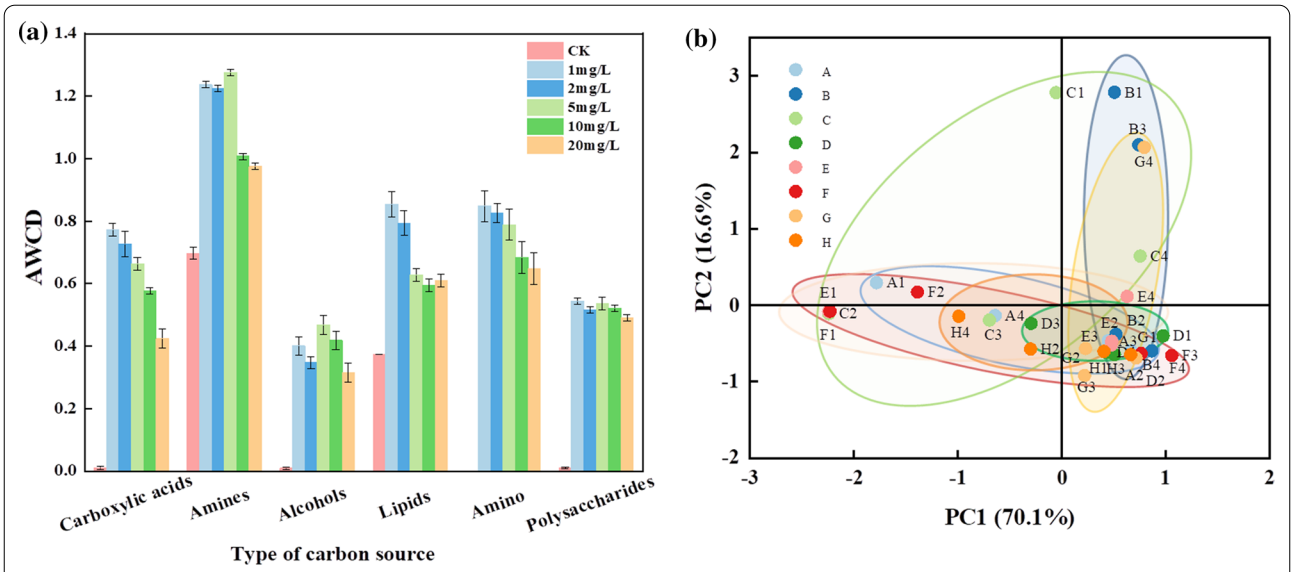

Fig. 7 **a** Utilization of different carbon sources by GD-1 and **b** principal component analysis of carbon source utilization by GD-1 under OFL stress

Comprehensive analysis showed that under OFL stress, itaconic acid, Tween 80, and L-aspartic acid were all significantly positively correlated with PC1, and significantly negatively correlated with PC2, indicating that under antibiotic stress, these carbon sources may be related to microbial bacteria. The growth and metabolism of the group are closely related. The compounds may enter cells through biofilm or as carbon source substrates, affecting cell metabolism and, thereby, increasing the cellular biomass and promoting the degradation of pollutants. Compared with Tween 20, Tween 80 causes less structural interference, has a weaker binding affinity and fewer binding sites and is thus a preferred hydrophilic surfactant [51, 52]. It is irritating to cell membranes and prone to oxidative degradation. For example, it can be used as a substrate in the separation of lipolytic microorganisms; Studies have shown that Tween 80 has different effects on extracellular enzymes produced by microorganisms during composting, including increasing the activities of amylase, CMCase, and xylanase, thereby accelerating the degradation of organic matter and improving composting efficiency [53]. There are also studies showing the addition of Tween 80 as a co-metabolic substrate to enhance the degradation of m-xylene by microecological bacteria to obtain the best degradation performance [54]. L-aspartic acid is an amino acid substrate, which can be metabolized through amino acid pathways. It can enter the metabolic pathways of microorganisms to promote growth and metabolism. Itaconic acid is an unsaturated dibasic organic acid, and microorganisms can use not only itaconic acid as a carbon source substrate for general metabolic and reproductive activities but it can also form oxalosuccinic acid or intermediate metabolites and can enter the tricarboxylic acid (TCA) cycle to generate NADH, providing electrons for redox reactions [55]. The bacterial consortium XG also shows great potential for bioremediation of ciprofloxacin-contaminated environments in the presence of the co-substrate itaconic acid [42]. Thus, it is apparent that the selection of itaconic acid, L-aspartic acid, and Tween 80 as the co-metabolic carbon sources for the degradation of OFL by the flora can improve the metabolic activity of the functional flora, allowing efficient degradation of OFL. Indeed, studies have shown that the selection of substances that are easily decomposed and utilized by microorganisms as co-metabolism substrates, such as easily available carbohydrates and amino acids, can facilitate the rapid reproduction of microorganisms, increase their biomass, and enhance their metabolic activity [56]. Furthermore, the addition of antibiotics can also alter the absorption of nutrients, such as amino acids, by the microbial community by affecting the composition of microbial colonies [23]. A recent study found co-metabolic degradation of ciprofloxacin by bacterial colony XG resulted in the removal of essential functional groups from the parent compound, resulting in the formation of metabolites with less bioactive potency. Co-metabolism of ciprofloxacin by bacterial flora XG with various carbon sources resulted in degradation of ciprofloxacin by up to 63% [42,58] . The enzymatic mechanisms used during microbial community degradation after the addition of co-metabolizing carbon sources are unclear. Nevertheless, our experimental results suggest that added carbon sources and antibiotics can interact with each other to

Table 1 Load diagrams of 31 carbon sources on the first and second principal components

Type of carbon source	Substrate	Number	PC1	PC2
Polysaccharides	D-xylose	B2	0.522	− 0.381
	D-Lactose	H1	0.406	− 0.604
	β-Methyl D-glucoside	A2	0.668	− 0.597
	Glucose-1-Phosphate	G2	0.229	− 0.566
	a-cyclodextrin	E1	− 2.241	− 0.082
	Glucose	F1	− 2.229	− 0.079
	D-Cellobiose	G1	0.715	− 0.690
Amino acids	L-Arginine	A4	− 0.640	− 0.136
	L-Asparagine	B4	0.866	− 0.598
	L-Phenylalanine	C4	0.754	0.644
	L-Serine	D4	0.433	− 0.618
	L-Threonine	E4	0.629	0.115
	Glycosyl-L-glutamic acid	F4	0.764	− 0.631
Esters	methyl pyruvate	B1	0.507	2.787
	Tween 40	C1	− 0.054	2.782
	Tween 80	D1	0.975	− 0.402
	D-galactonic acid gamma lactone	A3	0.447	− 0.465
Alcohols	I-erythritol	C2	− 2.239	− 0.111
	D-Mannitol	D2	0.508	− 0.645
	D, L-a-glycerol	H2	− 0.299	− 0.572
Amines	phenethylamine	G4	0.794	2.066
	putrescine	H4	− 0.991	− 0.145
	N-Acetyl-D-Glucamine	E2	0.481	− 0.470
Hydroxy acids	D-galacturonic acid	B3	0.739	2.099
	D-Glucosamine	F2	− 1.387	0.173
	2-Hydroxybenzoic acid	C3	− 0.695	− 0.194
	4-Hydroxybenzoic acid	D3	− 0.295	− 0.237
	R-Hydroxybutyric acid	E3	0.475	− 0.523
	Itaconic acid	F3	0.629	− 0.657
	A-butanone acid	G3	0.220	− 0.919
	D-malic acid	H3	0.663	− 0.646

influence the metabolism of the microbial community and thus the effectiveness of the microbial community in degrading the contaminants. This also suggests that the presence of co-metabolized substrates should be given high priority for in situ biodegradation of OFL. Clearly, further studies based on RNA (meta-transcriptomic analysis) are required for detailed functional analyses of the OFL-degrading microbial community.

Conclusions

(1) Under the stress of OFL, the changes in microbial communities were related to the addition of co-metabolic carbon sources, with the addition of amino acid carbon sources leading to significant changes in the microbial communities. Moreover, the addition of carbon sources and OFL interactively influenced the metabolism of the microbial community. Compared with the first acclimation stage, the rate of OFL degradation by the efficient OFL-degrading bacteria increased by 87%. The community composition was found to be Oligotrophic Bacteria (33.33%), *Alcaligenes* (27.24%), *Achromobacter* (22.39%), *Sphingobacterium* (9.84%), and *Micromonospora* (1.45%). After domestication, *Alcaligenes* and *Stenotrophomonas* became the dominant genera. Functions associated with membrane transport were found to be enriched in the microbial communities.

(2) Significant differences in carbon source utilization by the flora were seen under different OFL concentrations. Low OFL concentrations promoted the utilization of amines, amino acids, esters, and carboxylic acids by the flora, with microorganisms able to use these carbon sources showing the fastest growth rates. In the presence of OFL, itaconic acid,

Tween 80, and L-aspartic acid were found to stimulate functional microbial isomerization, improve substrate co-metabolism, and increase biomass, providing a novel detoxification strategy that would be most likely to provide a superior environment for degradation. This result also indicated that the concentration of OFL directly affected the growth and metabolic patterns of microbial communities, and different co-metabolic mechanisms led to the production of different metabolites, which in turn promoted the synergistic metabolism and activity of the flora, ultimately achieving efficient biodegradation of OFL. This is a guideline for subsequent studies on the co-metabolic mechanisms of microbial communities exposed to pollution.

(3) This study provides valuable information and new insights on developing bioremediation strategies for the removal of OFL contamination in natural environments. In addition, the results of the study on the metabolic diversity of functional microbiota with the addition of specific carbon sources for co-metabolism also extend existing knowledge on functional microorganisms in soil ecosystems affected by OFL pollution.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12302-022-00691-3.

Additional file 1: Figure. S1 The AWCD of GD-1 on Biolog-ECO plate treated with different concentrations of OFL. **Table. S1** The proportion of the main elements of the sample.

Author contributions

YL and NS designed and conducted the experiments. JZ and NS compiled and analyzed the output data, designed and wrote the first version of the manuscript, and conceived and supervised the project. ST, YP and YZ managed the funding acquisition. All authors read and approved the final manuscript.

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Availability of data and materials

Detailed information and additional data are available in the supplement (Additional file 1). Further information will be provided upon request from the corresponding author.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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