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Adaptation responses of *Dunaliella* sp. and its natural symbiotic bacteria to sulfamethoxazole

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ABSTRACT

Microalgal-bacterial consortia provide a sustainable, low-cost solution for antibiotic-contaminated wastewater remediation. Microalgae cultivation typically involves natural symbiotic bacteria, which are often overlooked. To fill this gap, this study evaluated the adaptive response of *Dunaliella* sp. and its natural symbiotic bacteria to sulfamethoxazole (SMX) through cultivation experiments and 16S rDNA sequencing. The results demonstrated strong SMX tolerance but limited removal efficiency by the consortium. The adaptive responses were characterized by increased bacterial diversity and connectivity, with quorum sensing bacteria-driven community changes and biofilm formation playing a critical role in this process. *Dunaliella* sp. exhibited strong antioxidant capacity (with catalase playing a key role) and increased extracellular polymeric substances secretion, which not only contributed to its tolerance but also promoted microalgae-bacteria interactions, enhancing consortium adaptability. These findings strengthen our understanding of the responses of natural symbiotic bacteria to antibiotics and advance the development of microalgae-bacteria symbiosis technology for wastewater treatment.

1. Introduction

The demand for mariculture continues to expand with the decline in wild marine fishery resources. Globally, antibiotic usage reached 93 million tons in 2017 and is projected to exceed 236 million tons by 2030, with 5.7 % used in aquaculture (Adenaya et al., 2023). However, antibiotic abuse leads to elevated residue levels, disrupting microbial communities, promoting antibiotic resistance genes (ARGs), and causing toxicity to aquatic organisms (Wang et al., 2022a, Peng et al., 2025). Sulfamethoxazole (SMX), one of the most widely used antibiotics in aquaculture, is typically detected in water bodies at concentrations ranging from 67.9 ng/L to 63.6 μg/L (Zhu et al., 2022). In shrimp farming ponds, SMX concentrations can reach up to 2.39 mg/L (Le and Munekage, 2004), and in pharmaceutical wastewater can also reach mg/L levels (Zhang et al., 2025). Even in natural seawater, the highest reported SMX concentration is 1454.2 ng/L (Niu et al., 2016). Traditional methods like adsorption, activated sludge, advanced oxidation, and electrochemistry face challenges including inconsistent efficiency, high costs, and the potential to promote the spread of ARGs (Lu

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et al., 2025). In contrast, microalgae-based biological treatment technologies are gaining attention for their low cost, sustainability, and environmental friendliness.

Microalgae are important components of aquatic ecosystems and have been shown to degrade antibiotics while removing nitrogen and phosphorus from wastewater (Chandel et al., 2022, Zhang et al., 2022a). However, most microalgae show obvious growth inhibition under the influence of moderate concentrations of SMX (Xiong et al., 2019b, Xiong et al., 2020, Zhang et al., 2022b), limiting their practical application. Recent studies suggest that introducing foreign microorganisms can enhance the adaptability and removal efficiency of microalgae to antibiotics. This improvement is attributed to symbiotic interactions, involving extracellular polymeric substances (EPS)-mediated adhesion, nutrient exchange, oxygen release, and alterations in bacterial community structure and function (da Silva Rodrigues et al., 2020, Hu et al., 2022, Wang et al., 2022b). However, consortia composed of microalgae and foreign bacterial communities exhibit significant uncertainty and instability.

In practice, microalgae are often cultivated as monocultures rather than pure cultures, naturally carrying symbiotic bacteria whose roles have been largely overlooked. Most research has focused on the combined effects of algae with foreign microorganisms, while the role of natural symbiotic bacteria has not been fully studied. To fill this knowledge gap, this study focused on a microalga from the genus *Dunaliella*, a unique group of halophilic, unicellular, eukaryotic microalgae widely distributed in marine and hypersaline environments (Wang et al., 2023). *Dunaliella* has shown high tolerance to adverse environments, including high salinity (Liska et al., 2004), heavy metals (Folgar et al., 2009), microplastics (Chae et al., 2019), and various organic pollutants (Quesnel et al., 2011, Xiong et al., 2016). However, few studies have explored its response to antibiotics (Kvíderová and Henley, 2005, Machado and Soares, 2019, Hom-Diaz et al., 2022), and no studies have investigated the impact of SMX on *Dunaliella* or its natural symbiotic bacteria.

This study investigated the adaptive responses of *Dunaliella* sp. and its natural symbiotic bacteria to different SMX concentrations by analyzing microalgal growth, nutrient utilization, antibiotic removal, stress response, and 16S rDNA sequencing. The objectives were to investigate *Dunaliella* sp.'s SMX removal efficiency and tolerance mechanisms, and explore the response and interactions of the natural symbiotic bacterial community under SMX stress. To our knowledge, this is the first study to investigate the impact of antibiotic stress on microalgae's natural symbiotic bacteria. These findings provide new insights into the application of algae-bacteria symbiosis technology in bioremediation and bioenergy production.

2. Materials and methods

2.1. Chemicals and microalgae

Tested SMX (purity ≥98 %, CAS No. 723–46–6) was purchased from Aladdin Biochemical Technology Co., LTD (Shanghai, China). Methanol and acetonitrile (HPLC grade) were purchased from Thermo Fisher Scientific Inc. (USA). Other reagents were analytical grade unless otherwise specified. The marine microalga *Dunaliella* sp. (SCSIO-45184) was obtained from the Guangdong Province Economic Microalgae Species and Quality Resource Database (China) and cultured in F/2 medium.

2.2. Microalgal-bacterial consortium culture and exposure experiment

The microalgal-bacterial consortium was cultured in simulated mariculture wastewater with SMX concentrations of 0, 1, and 10 mg/L for ten days. Although these concentrations exceed environmental levels, they are commonly used in algae-bacteria remediation screening studies to identify robust combinations and amplify their adaptive traits (Hu et al., 2022, Zhang et al., 2022a). Sterilised natural seawater was used as the basic culture medium supplemented with 4 mg/L nitrate (NO_3^--N), 2 mg/L ammonium (NH_4^+-N), and 0.5 mg/L phosphates (PO_4^{3-}-P) according to prior investigations. Glucose and sodium bicarbonate were added as carbon sources.

Experiments were conducted in triplicate using 1 L Erlenmeyer flasks, with an initial cell density of approximately 1.0×10^4 cells mL^{-1}. Cultures were maintained at 25 ± 1℃ under a 12 h light/12 h dark cycle and shaken three to four times daily. From day 0, culture suspensions were collected every two days to determine the biomass, pigments, nutrients, and SMX content. Additionally, EPS, activities of the five key antioxidant enzymes, and contents of glutathione (GSH) and malondialdehyde (MDA) were only measured on days 4 and 10.

2.3. Natural symbiotic bacteria exposure experiment

Natural symbiotic bacteria were cultured in 250 mL Erlenmeyer flasks (three replicates) under the same SMX and medium conditions as the consortium. Before incubation, the consortium was centrifuged at 4500 rpm for 15 min. The supernatant was discarded, and 0.5 % sterile saline was added to achieve thorough resuspension. *Dunaliella* sp. ruptured completely due to the absence of cell walls, leaving a natural symbiotic bacterial suspension for incubation (Fig. S1). Culture suspensions were collected every two days for nutrient analysis.

2.4. Physiological analyses

Microalgae were fixed with Lugol's solution and counted using a haemocytometer under a microscope. Total chlorophyll and carotenoid contents were determined at 665, 652, and 470 nm using a spectrophotometer (METASH UV-9000S, China), calculated as follows (Xiong et al., 2017):

$$\text{Chlorophyll a (Chla)} = 16.82\ A_{665} - 9.28\ A_{652}$$

$$\text{Chlorophyll b (Chlb)} = 36.92\ A_{652} - 16.54\ A_{665}$$

$$\text{Carotenoid} = (1000\ A_{470} - 1.91\text{Chla} - 95.15\text{Chlb})/225$$

EPS were collected by centrifugation (4500 rpm, 5 minutes, 4°C) and classified as slime EPS (Mao et al., 2022). Polysaccharides (PS) and proteins (PN) were determined using assay kits based on bicinchoninic acid and anthrone-sulfuric acid methods, respectively. GSH and MDA contents and the activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione S-transferase (GST), glutathione reductase (GR), and catalase (CAT) were detected using assay kits. The results were obtained using an ultra-micro spectrophotometer (BIO-DL Micro Drop, China). All kits, except for PS (Youxuan Biotechnology Co., Ltd., China), were sourced from the Nanjing Jiancheng Bioengineering Institute, China.

2.5. *Analysis of nutrients and SMX*

The concentrations of NO_3^--N, nitrite (NO_2^--N), NH_4^+-N, PO_4^{3-}-P, total nitrogen (TN), and total phosphorus (TP) were determined using a flow analyser (Skalar San++, Netherlands) according to the standard methods for seawater detection by the Ministry of Ecology and Environment of China. Method details are presented in Supplementary Material Table S1. Solid-phase extraction and high-performance liquid chromatography with fluorescence detection were used to analyze SMX content, as detailed in the Supplementary Materials Text S1.

2.6. *16S rDNA high-throughput sequencing of natural symbiotic bacteria*

On days 0, 4, and 10, microalgal-bacterial culture samples were collected. A 100 mL aliquot was centrifuged at 4500 rpm for 15 min, and the supernatant was separated. 0.5 % sterile saline was added to achieve thorough resuspension, facilitating lysis of the microalgae cells. The suspension and supernatant were filtered through a 0.22 μm acetate membrane and sent to Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) for DNA extraction, PCR amplification and high-throughput sequencing. After quality filtering, merging, clustering, and chimera removal, 1256,804 optimized sequences were obtained for analysis.

D4_0, D4_1, and D4_10 were blank, 1 mg/L, and 10 mg/L groups on day 4; D10_0, D10_1, and D10_10 were the same on day 10. Species classification and functional gene annotation were based on SILVA138/16S_bacteria and Kyoto Encyclopedia of Genes and Genomes databases. Raw sequencing data can be accessed from the National Centre for Biotechnology Information under accession number PRJNA1155904.

2.7. *Statistical analysis*

The standard curve correlation coefficient for each detection index exceeded 0.995, and 10 % random replicates were set up with a relative deviation of less than 2 %. Chemical indicators were analyzed using IBM SPSS Statistics 27 and microbial analysis was performed on the Majorbio platform. Treatment groups differences were compared using one-way ANOVA ($p < 0.05$). Results were visualized using Origin 2021 or Adobe Illustrator 2023.

3. Results

3.1. *Effect of SMX on the growth and pigment synthesis of Dunaliella sp*

The effects of SMX concentration on *Dunaliella* sp. growth are presented in Fig. 1. *Dunaliella* sp. initially grew rapidly, with growth

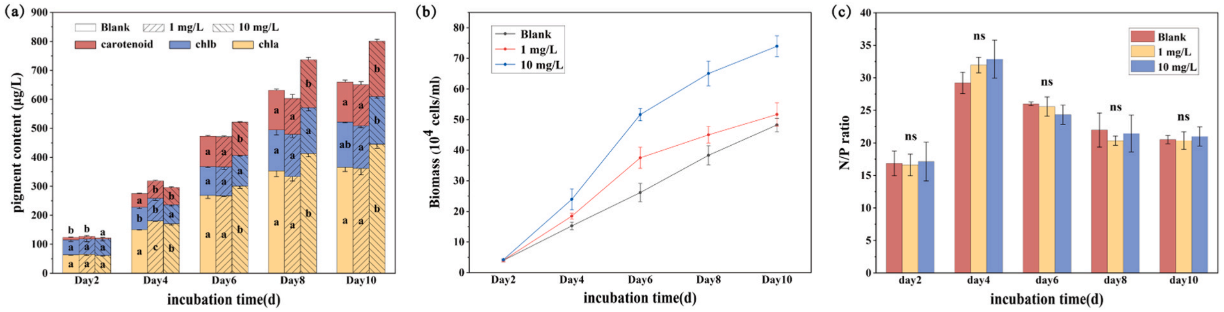


Fig. 1. (a) Trends in the contents of Chla, Chlb, and carotenoid; (b) Trends in the cell density of *Dunaliella* sp.; and (c) Trends in the N/P ratio within *Dunaliella* sp. cells under different SMX concentrations during 10 d of cultivation. Error bars represent standard deviation (n = 3). Different letters indicate significant differences ($p < 0.05$) between treatments on the same day and the same letters or “ns” indicate no differences.

slowing after day 4 and no inhibition observed. From day 4 onward, the 10 mg/L group showed significantly higher biomass than other groups, reaching 1.53 times that of the blank group by the end of the experiment. Pigment content followed a similar trend, with significantly higher Chla and carotenoids contents observed in the 10 mg/L group from day 6.

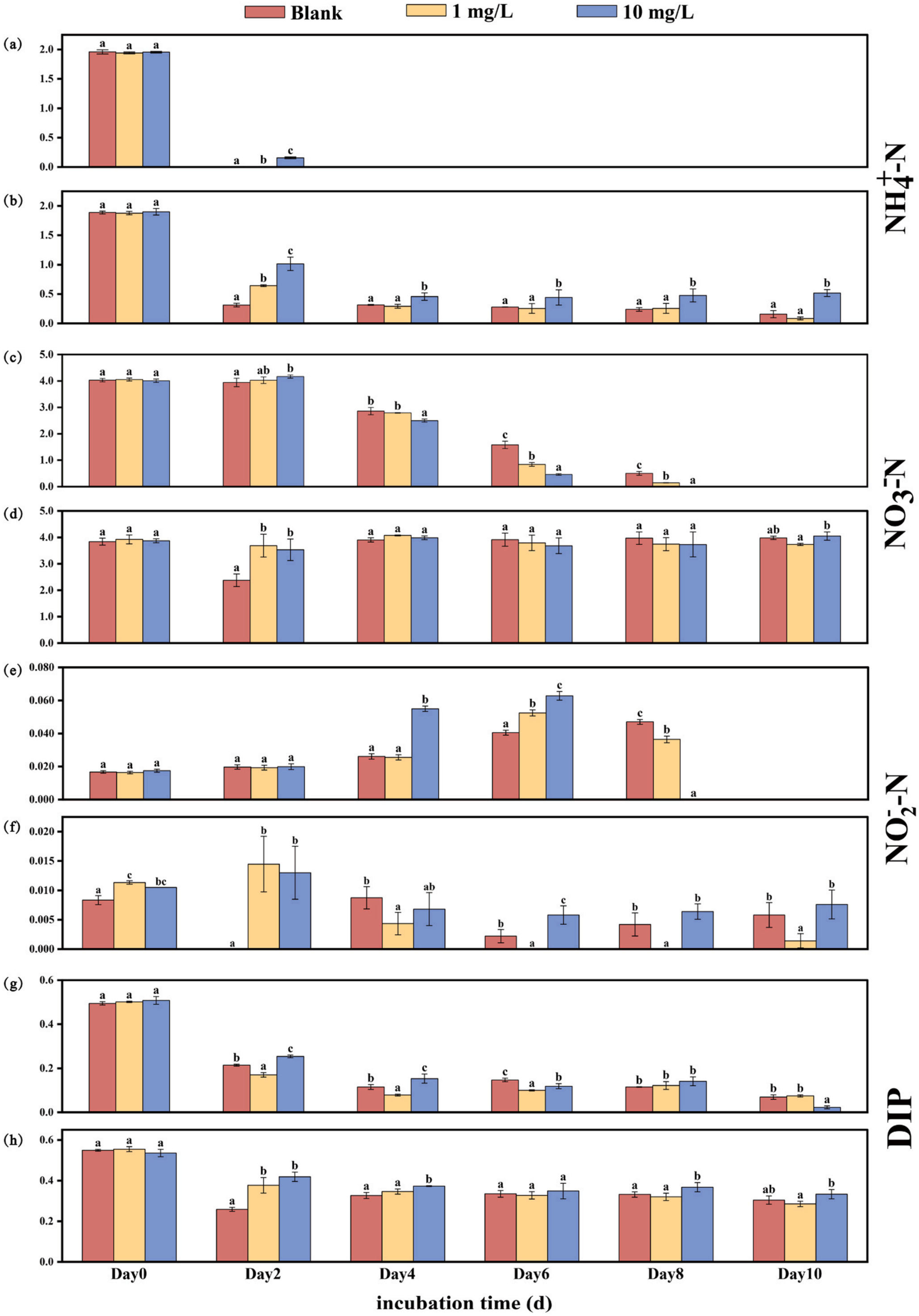


Fig. 2. Trends in nutrient utilization by microalgal-bacterial consortium (a, c, e, g) and natural symbiotic bacteria alone (b, d, f, h) under different SMX concentrations during 10 d of cultivation. Error bars represent standard deviation (n = 3). Different letters indicate significant differences ($p < 0.05$) between treatments on the same day and the same letters indicate no differences.

The N/P ratio in *Dunaliella* sp. (Fig. 1c) changed as follows: under nutrient sufficient conditions on day 2, the N/P ratio was close to the Redfield ratio (N/P = 16). By day 4, under phosphorus limitation, the N/P ratio rose above 30, higher in SMX-treated groups than the blank group. Subsequently, the N/P ratio gradually decreased and stabilised at approximately 20.

3.2. Nutrient utilisation of *Dunaliella* sp. and its natural symbiotic bacteria under SMX stress

The effect of SMX concentration on nutrient utilization by the microalgal-bacterial consortium is presented in Fig. 2(a, c, e, g). NH_4^+-N was consumed first, nearly depleted by day 2 except for the 10 mg/L group. NO_3^--N contents were stable initially, with slight increases in SMX-treated groups, but rapidly declined from day 4 and were eventually completely removed. Additionally, from day 4 onward, SMX-treated groups significantly accelerated NO_3^--N consumption, with NO_3^--N in the 10 mg/L group fully depleted by day 8. In contrast, NO_2^--N accumulation increased with higher SMX concentrations, but was consumed once NO_3^--N and NH_4^+-N were completely absorbed. Unlike nitrogen, PO_4^{3-}-P was not completely utilized, and its concentration exhibited a fluctuating reduction following an initial rapid decline.

Changes in the nutrient utilization patterns of natural symbiotic bacteria under SMX stress after separation from *Dunaliella* sp. are presented in Fig. 2(b, d, f, h). The bacteria presented high NH_4^+-N utilization efficiency, with the blank group consuming 83.4 % by day 2 and reaching 91.7 % eventually, but efficiency suppressed significantly with increasing SMX concentrations. NO_3^--N in the blank group was initially consumed but rapidly released, while levels in other groups remained stable. NO_2^--N also showed accumulation, but its contents were negligible compared to those in the microalgal-bacterial consortium. PO_4^{3-}-P utilization followed a pattern similar to ammonia but with slightly lower efficiency, peaking at 52.9 % in the blank group.

3.3. SMX removal efficiency and its effect on MDA and EPS content

The SMX residue rates at different SMX concentrations are presented in Fig. 3a, with values of 84.71 % and 90.20 % for the 1 and 10 mg/L groups, respectively. On day 4, MDA content (Fig. 3b) in the 10 mg/L group was 7.68 times higher than in the blank group but returned to normal by day 10. Additionally, on day 4, the 10 mg/L group showed significantly higher PS and PN contents in the slime

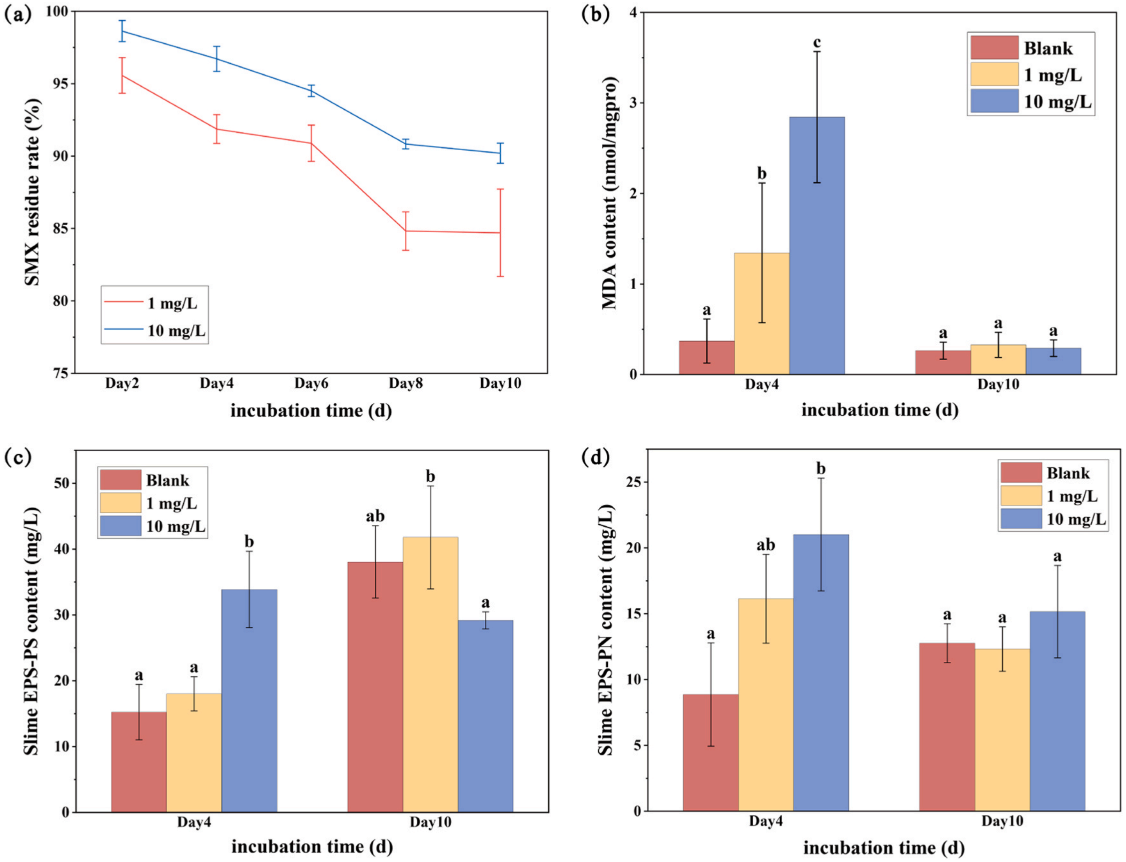


Fig. 3. Trends in (a) SMX residue rate, (b) MDA content in *Dunaliella* sp. cells, (c) PS content in slime EPS and (d) PN content in slime EPS under different SMX concentrations during incubation periods. Error bars represent standard deviation (n = 3). Different letters indicate significant differences ($p < 0.05$) between treatments on the same day and the same letters indicate no differences.

EPS (Fig. 3c-d) than those in blank group. By day 10, PS and PN contents in the 10 mg/L group had decreased.

3.4. *Response of antioxidant system to SMX*

Activity of antioxidant system in *Dunaliella* sp. are shown in Fig. 4a. On day 4, CAT, SOD, and GR activities increased with SMX concentration. The CAT activity in the 10 mg/L group was significantly higher than that in the blank group, while GST and GPX activities were significantly lower. By day 10, the CAT activity remained elevated in the 10 mg/L group, GST continued to decrease and GR activity reversed. SOD and GPX activities, and GSH content showed almost no differences among the treatment groups.

PCA results (Fig. 4b-c) showed that the sample points on day 4 were more clustered within groups and exhibited clearer separation between groups compared to day 10. On day 4, PC1 and PC2 explained 54.1 % and 18.5 % of the total variance, respectively. MDA, CAT, GR, PS, and PN were positively correlated with SMX concentrations, while GST and GPX showed negative correlations. On day 10, PC1 and PC2 explained 38.9 % and 24.6 % of total variance, respectively. Notably, the effect of MDA was negligible. CAT and PN remained positively correlated with SMX concentration, while the response of other indicators to SMX concentration weakened.

3.5. *16 s rDNA analysis on natural symbiotic bacteria of Dunaliella sp*

3.5.1. *Natural symbiotic bacteria community structure analysis*

The alpha diversity index was used to assess the changes in diversity of natural symbiotic bacterial communities under different SMX concentrations (Fig. 5a). Significantly higher Shannon's index and lower Simpson's index values with increasing SMX concentrations, implied increased biodiversity. Beta diversity (Fig. 5b), analysed by principal coordinate analysis (PCoA), revealed that the

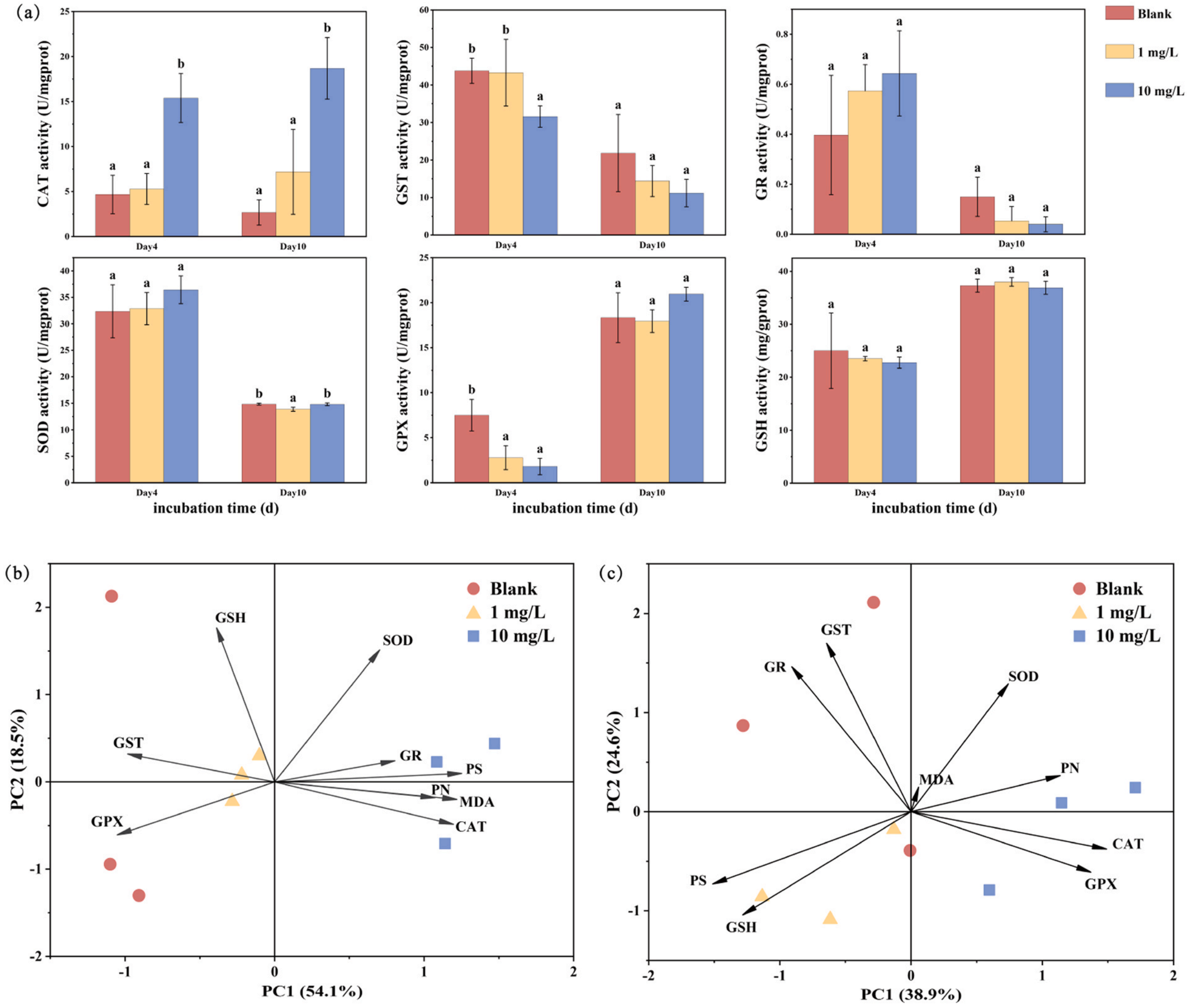


Fig. 4. Under different SMX concentrations, (a) trends in the activities of antioxidant enzymes in *Dunaliella* sp. cells, and PCA of various physiological indicators related to the antioxidant system and EPS on day 4 (b) and day 10 (c).

bacterial composition in stimulated mariculture wastewater differed from the initial F/2 medium, and SMX significantly affected it as early as day 4.

The natural symbiotic bacterial community of *Dunaliella* sp. consisted of four phyla, 38 genera, and 40 species (15 identified). Only one genus disappeared during the incubation, with 31 genera remaining in all treatment groups (Fig. 5c). Bacterial composition results (Fig. 5d) showed that the relative abundance of dominant genera increased in stimulated mariculture wastewater compared to the F/2 medium but were evidently inhibited as SMX concentration increased. *Methylophaga* and *Labrenzia* had considerably lower relative abundances in the 10 mg/L group than in the blank group, while *Ponticoccus* and *Mameliella* increased. LDA Effect Size (LEfSe) analysis (LDA threshold = 4, Fig. 5e) identified 12 significantly different genera, mostly enriched in *Alphaproteobacteria* and derived from moderate abundance genera in day 10 samples from the 10 mg/L group.

One-way correlation networks were used to analyse the correlations between species and the results are presented in Fig. 6a. *Proteobacteria* remained the core phyla, while the core genera shifted from *Methylophaga* and *Labrenzia* to *Ponticoccus* and *Mameliella* as SMX concentration increased. The number of linkages markedly increased with both the incubation period and SMX concentration. For example, on day 4, the 10 mg/L group had 286 linkages, significantly higher than the blank (210) and 1 mg/L (198) groups. By day 10, linkages further increased to 378 (10 mg/L) and 360 (1 mg/L), compared to 236 in the blank group. Additionally, the positive correlation ratio (Fig. 6b) in the 10 mg/L group (53.5 % on day 4 and 54.0 % on day 10) was higher than in the blank (45.6 % and 46.6 %) and 1 mg/L (44.8 % and 49.4 %) groups. These results indicate that the connectivity within the natural symbiotic bacterial community increased under SMX stress.

3.5.2. *Functional predictions*

The BugBase phenotype prediction results for natural symbiotic bacteria under different SMX concentrations are presented in Fig. 7a. The relative abundance of gram-negative and facultative anaerobic bacteria increased with SMX concentrations. Notably, biofilm-forming phenotypes also increased significantly, while the potentially pathogenic phenotypes decreased significantly (Fig. 7b). Additionally, KEGG pathway analysis (Fig. 7c) revealed that metabolic pathways had the highest relative share of 74.8 % at level 1, while the trends at level 2 were essentially consistent across all groups on days 4 and 10. Furthermore, level 2 comparisons revealed significant changes in bacterial physiological processes with increasing SMX concentrations. Specifically, pathways related to xenobiotic degradation and metabolism, amino acid metabolism, and lipid metabolism increased, while those associated with sugar biosynthesis and metabolism, energy metabolism, and cellular motility decreased.

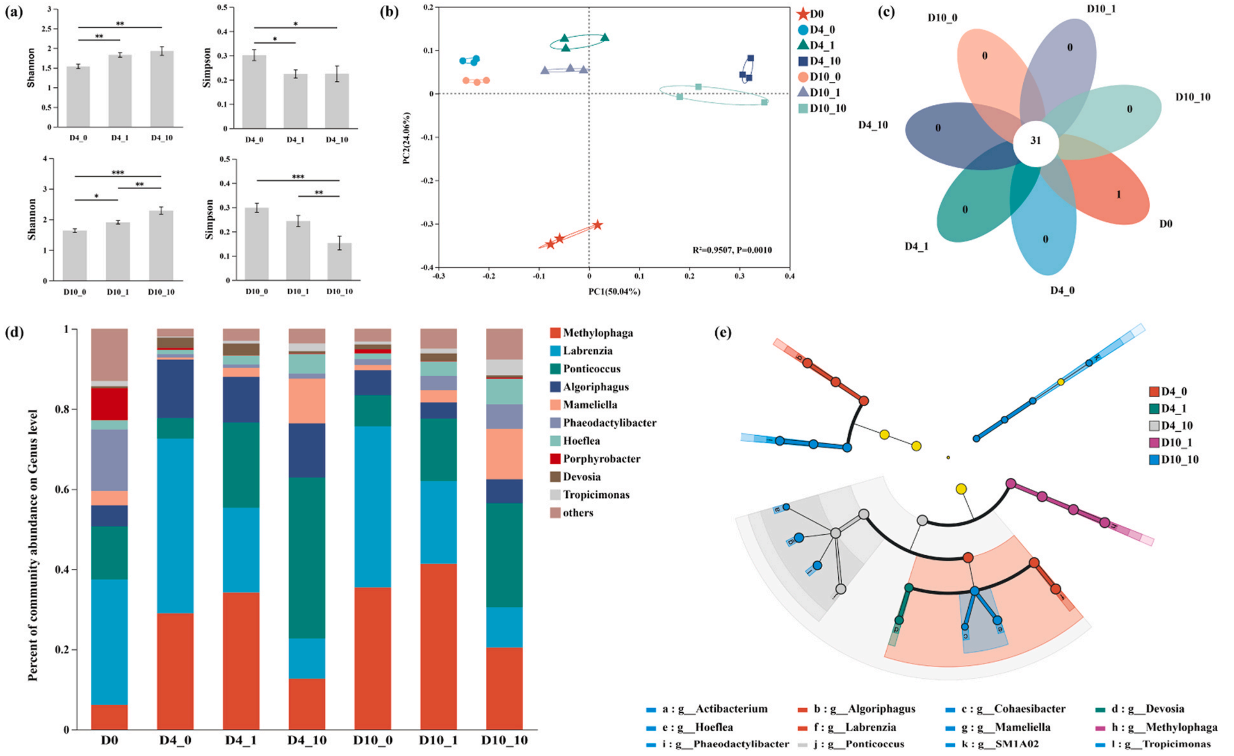


Fig. 5. Analysis of the composition of natural symbiotic bacterial communities under different SMX concentrations. (a) Shannon and Simpson indices of OUT level, (b) PCoA on OUT level, (c) Venn diagram of the genera, (d) Relative abundance of the genus level and (e) LEfSe multilevel species hierarchical cladogram. * , * *, and * ** indicate different significant differences $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

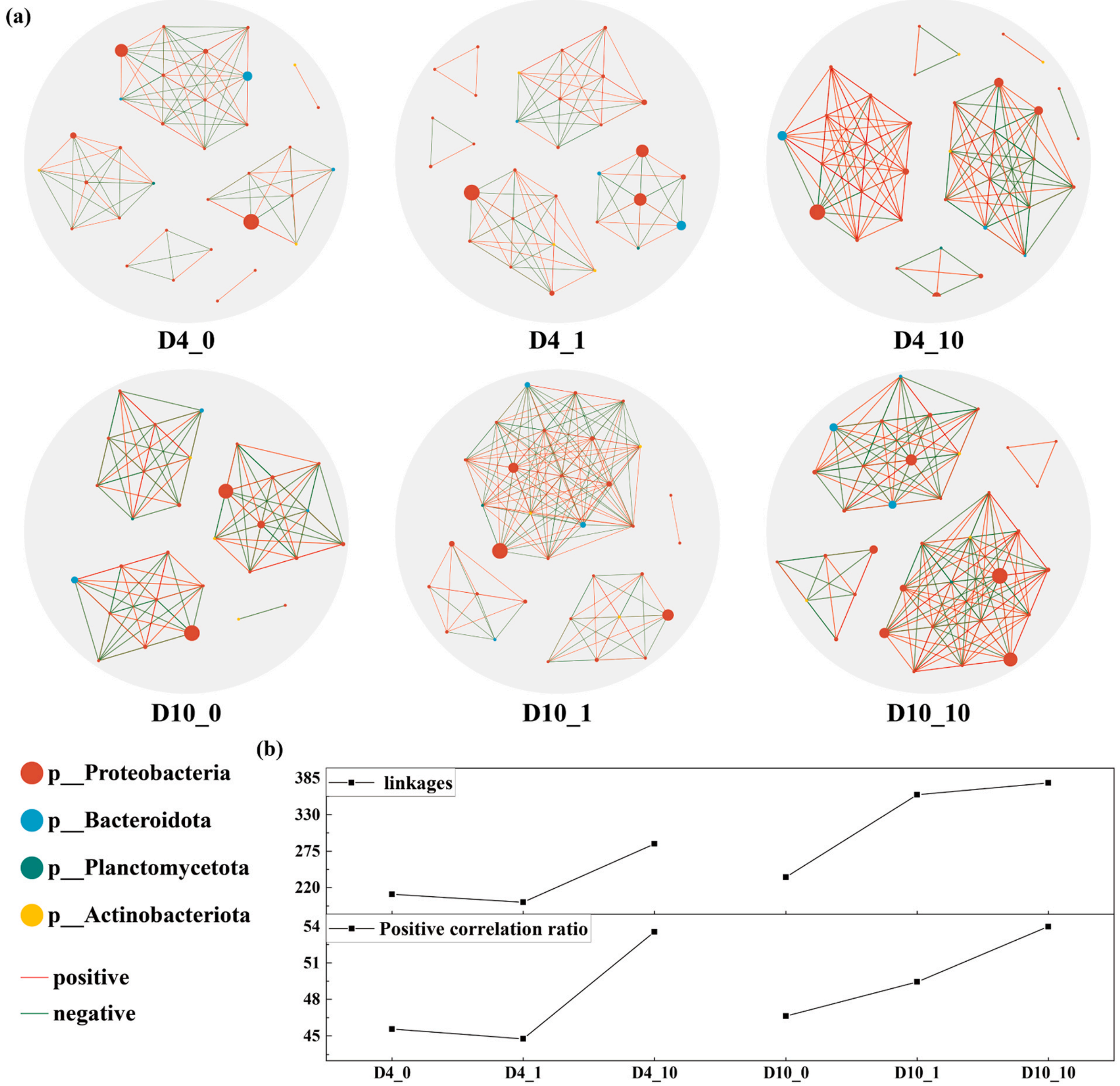


Fig. 6. One-way correlation network analysis of natural symbiotic bacterial communities at different time points under varying SMX concentrations. The nodes represent the species at the phylum level and linkages indicate a correlation between species ($|r| > 0.8$).

4. Discussion

4.1. SMX stress enhances Dunaliella sp. biomass via reduced bacterial competition and adaptive responses

This study confirmed that *Dunaliella* sp. showed a strong adaptation to SMX, as evidenced by their biomass, pigment content, and nutrient utilization efficiency. The microalgal biomass increased likely resulted from SMX restricting bacterial nutrient competition (Zhou et al., 2017), further supported by natural symbiotic bacterial isolation and culture experiments showing SMX inhibited bacterial ammonia and phosphate utilization. Since no nitrifying bacteria were detected in the natural symbiotic bacteria, NH_4^+-N consumption was primarily attributed to bacterial assimilation (Kuypers et al., 2018). The order of nitrogen utilization by *Dunaliella* sp. was consistent with that of most microalgae: NH_4^+-N > NO_3^--N > NO_2^--N (Perez-Garcia et al., 2011). Due to their competitive relationship, SMX restricted bacterial NH_4^+-N utilization, resulting in NH_4^+-N accumulation and promoting early biomass buildup in *Dunaliella* sp. Additionally, excess NH_4^+-N and SMX may have suppressed nitrate reductase and nitrite reductase activities in microalgal cells, leading to a stagnation in NO_3^--N consumption (Su, 2021). Once the microalgae adapted, the early accumulated biomass became a key driver of subsequent growth, enabling rapid NO_3^--N utilization in the later phase.

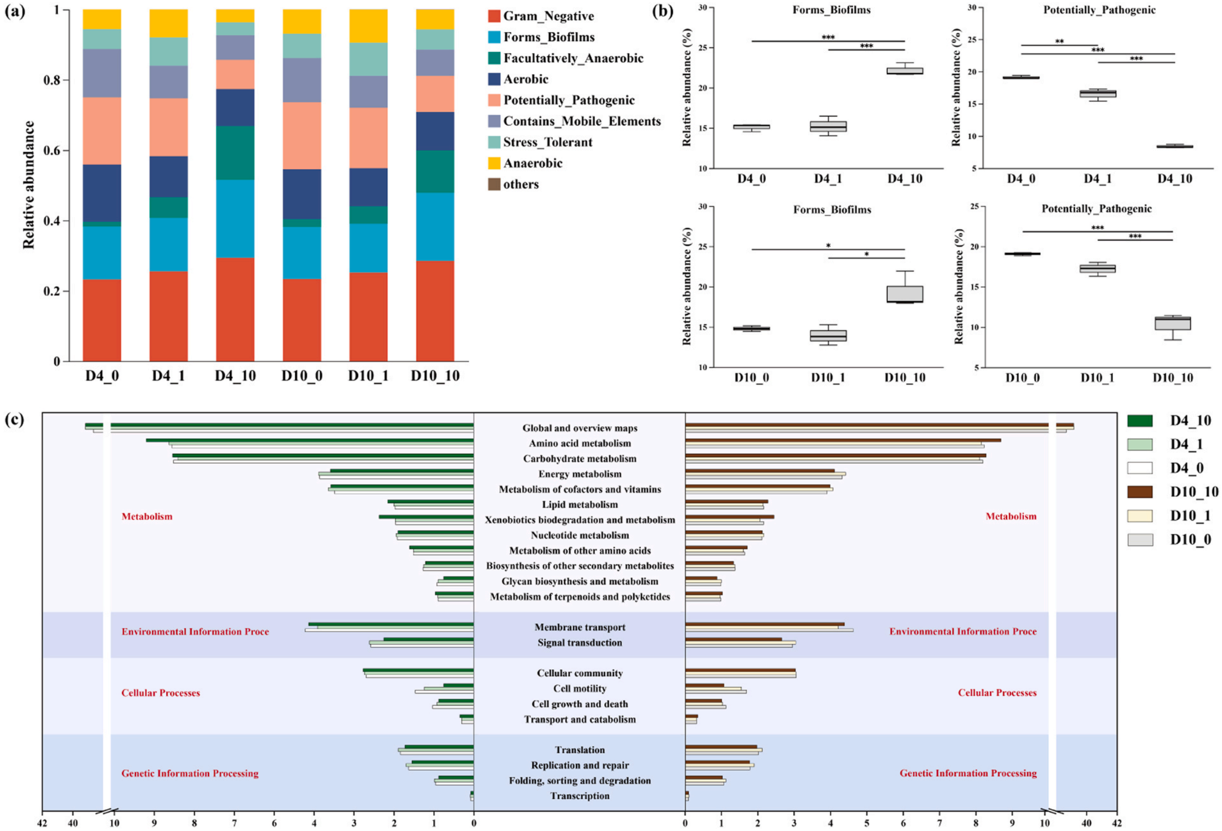


Fig. 7. Analysis of BugBase phenotype prediction (a), comparison of phenotypic differences (b), and KEGG pathway on level 1 and level 2 (c) of natural symbiotic bacteria communities under different SMX concentrations. * , * *, and * ** indicate different significant differences $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

In addition, bacteria can cause diseases in microalgae, such as gall formation (Ashen Jon and Goff Lynda, 2000), bleaching disease (Zozaya-Valdés et al., 2017), and hole-rotten disease (Wang et al., 2008). The reduced abundance of pathogenic phenotypes in natural symbiotic bacteria under SMX exposure may further contribute to the increased biomass. Furthermore，pigments content in *Dunaliella* sp. strongly correlated with biomass and can be influenced by oxidative stress and photosynthetic processes under abiotic stress (Chen et al., 2025). Chla can participate in the removal of reactive oxygen species in chloroplasts (Kasahara et al., 2002), whereas carotenoids can effectively quench excited chlorophyll, thereby preventing stress-induced damage to the photosynthetic system (Paliwal et al., 2015). Photosynthesis is the primary pathway for pigments production. Studies have found that at high concentrations (5 mg/L), SMX still can enhance photosynthesis in *Chlorella vulgaris* biofilms by promoting nitrogen uptake (Sun et al., 2025). In this study, the increased N/P ratio in *Dunaliella* sp. cells under SMX exposure also indicates enhanced nitrogen uptake, which may reflect improved photosynthesis and could be related to enhanced extracellular protein secretion and synthesis of defensive proteins (Gao et al., 2016). These findings suggest that SMX stress induced adaptive responses and enhanced photosynthesis in *Dunaliella* sp., stimulating growth. However, the molecular mechanisms behind this remain to be further explored.

4.2. *Dunaliella sp.* exhibits strong tolerance but poor removal ability to SMX

The excellent adaptability of *Dunaliella* sp. to high SMX concentrations is noteworthy. Excessive EPS production under stress is a key microbial defense strategy against toxic chemicals (Wang et al., 2021). Microalgae of the genus *Dunaliella* often increase EPS secretion in response to pollutant stress (Folgar et al., 2009, Wang et al., 2023). PS and PN are essential components of EPS due to their unique structures and functions (Wang et al., 2019). This study also observed that *Dunaliella* sp. produced significantly more PS and PN during the initial response to SMX, indicating that EPS enhancement is one of the key mechanisms for adapting to SMX stress. Interestingly, as cultivation progressed, the PS and PN content in the SMX-treated group declined.

EPS includes soluble EPS and bound EPS (Mao et al., 2022). Soluble EPS exhibits weaker interactions with cells and is more environmentally sensitive (Li et al., 2025). In bound EPS, PN can support bacterial aggregation, cell-to-cell signaling, and structural stability, while PS can promote microbial adhesion and biofilm formation (More et al., 2014). Additionally, EPS can enhance the conversion between inorganic and organic carbon, thereby supporting the growth of heterotrophic organisms and stabilizing the microalgae-bacteria consortium (Zhou et al., 2024). The increased diversity and connectivity of the natural symbiotic bacterial community may benefit from changes in EPS properties. The decrease in EPS content may be related to changes in its properties, potentially enhancing interactions between *Dunaliella* sp. and its natural symbiotic bacteria.

The antioxidant system is also vital for microalgae adapting to antibiotic stress (Xiao et al., 2024). The intracellular antioxidant system removes excess ROS through coordinated enzymatic and non-enzymatic reactions. SOD, CAT, and GPX catalyze the conversion of O_2^- to H_2O_2 and its breakdown into water (Gill and Tuteja, 2010), while GST removes harmful electrophilic substances (Zhang et al., 2022b). MDA is an important biomarker for the assessment of cellular oxidative damage (Zhang et al., 2022b). Changes in MDA content show that *Dunaliella* sp. is sensitive to short-term acute SMX damage but has strong recovery capabilities. Correspondingly, CAT activity rapidly and significantly increased and maintained high levels, indicating its crucial role in *Dunaliella* sp.'s adaptation to SMX stress. It is a common response in many microalgae under antibiotic stress (Chen et al., 2020b, Chen et al., 2025), and a study indicates that CAT is vital for enhancing SMX adaptability in acclimated *C. vulgaris* (Zhang et al., 2022b), suggesting CAT activity as a potential biomarker for assessing microalgal adaptability to antibiotics.

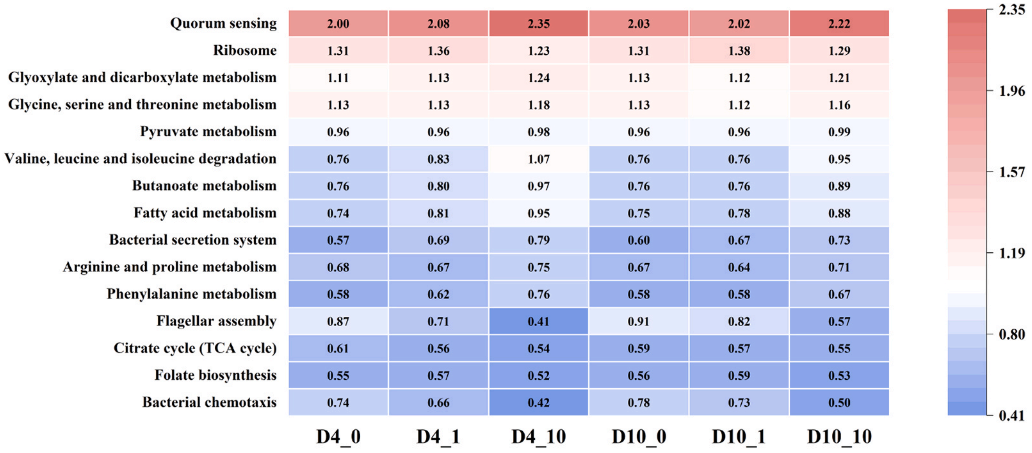
GSH is essential for maintaining the cellular reduced state, serving as a substrate for both GPX and GST, while its balance is regulated by GR (Gill and Tuteja, 2010). By day 4, increased GSH consumption enhanced GR activity, while GSH primarily adapted to SMX stress through non-enzymatic pathways, as SMX inhibited GST and GPX. By day 10, reduced SOD and GR activities and increased GSH content indicated the recovery of *Dunaliella* sp.'s antioxidant system, consistent with decreased MDA content.

Dunaliella sp. showed good adaptation to high SMX concentrations, but its removal efficiency was lower than that of most microalgae, such as *Nannochloris sp.* (32 %; (Bai and Acharya, 2016)), *Monoraphidium contortum* (42.3 %; (Costa-Ramos et al., 2025)), and *Scenedesmus obliquus* (16–29 %; (Xiong et al., 2019a)). Currently, microalgae of the genus *Chlorella* has shown the highest SMX removal efficiency, with wild *Chlorella vulgaris* achieving 76 % removal and an acclimated strain reaching 92.5 % (Zhang et al., 2022b). Microalgae remove antibiotics mainly through biosorption, bioaccumulation, and biodegradation (Xiao et al., 2024). Biosorption is a key step, and the cell wall plays a crucial role in this process. The diverse functional groups on the cell wall can adsorb various pollutants, and the cell wall and EPS exhibit coordinated effects in pollutant removal (Naveed et al., 2025). The study found that *Dunaliella tertiolecta* and the cell wall-free mutant of *Chlamydomonas reinhardtii* formed smaller aggregates under nanoparticle exposure compared to cell wall-containing microalgae (Oukarroum et al., 2012, Perreault et al., 2012). The absence of a cell wall likely weakens the biosorption capacity of *Dunaliella* sp., resulting in lower SMX removal efficiency.

In addition, The absence of a cell wall is not necessarily linked to increased sensitivity in microalgae (Ge et al., 2022). Therefore, the cell membrane undoubtedly plays a more critical role in cell wall-free microalgae. The increase in MDA content indicates damage to the structure and function of *Dunaliella* sp.'s cell membrane, which may also affect its interaction with SMX. Microalgal surfaces are usually negatively charged (Günan Yücel et al., 2021), whereas SMX exists primarily in a stable anionic form at $pH > 5.7$ (Wang and Wang, 2018). Under seawater conditions ($pH \sim 8.0$), SMX is likely to experience electrostatic repulsion. Antibiotics with higher lipophilicity (log Kow) are more easily adsorbed by microalgae, while SMX, with a log Kow of 0.89, exhibits strong hydrophilicity ($\log Kow < 1$) (Wang et al., 2022b). Thus, antibiotic removal efficiency is also influenced by *Dunaliella* sp.'s cell membrane characteristics, but deeper mechanisms need analysis via scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR).

4.3. Sulfamethoxazole alters the composition, connection and function of natural symbiotic bacterial communities

SMX is a broad-spectrum antibiotic that inhibits bacterial growth by competitively inhibiting dihydrofolate synthase and disrupting folate biosynthesis (Rodrigo et al., 2022). Functional prediction (Fig. 8) revealed reduced abundances of folate biosynthesis, ribosomes, flagellar assembly, bacterial chemotaxis, and the TCA cycle under SMX stress, all critical for bacterial growth, reproduction, and motility, and common targets of antibiotics (Xiu et al., 2017, Zhang et al., 2021, Yu et al., 2022, Xu et al., 2024). Consistent with



	D4_0	D4_1	D4_10	D10_0	D10_1	D10_10
Quorum sensing	2.00	2.08	2.35	2.03	2.02	2.22
Ribosome	1.31	1.36	1.23	1.31	1.38	1.29
Glyoxylate and dicarboxylate metabolism	1.11	1.13	1.24	1.13	1.12	1.21
Glycine, serine and threonine metabolism	1.13	1.13	1.18	1.13	1.12	1.16
Pyruvate metabolism	0.96	0.96	0.98	0.96	0.96	0.99
Valine, leucine and isoleucine degradation	0.76	0.83	1.07	0.76	0.76	0.95
Butanoate metabolism	0.76	0.80	0.97	0.76	0.76	0.89
Fatty acid metabolism	0.74	0.81	0.95	0.75	0.78	0.88
Bacterial secretion system	0.57	0.69	0.79	0.60	0.67	0.73
Arginine and proline metabolism	0.68	0.67	0.75	0.67	0.64	0.71
Phenylalanine metabolism	0.58	0.62	0.76	0.58	0.58	0.67
Flagellar assembly	0.87	0.71	0.41	0.91	0.82	0.57
Citrate cycle (TCA cycle)	0.61	0.56	0.54	0.59	0.57	0.55
Folate biosynthesis	0.55	0.57	0.52	0.56	0.59	0.53
Bacterial chemotaxis	0.74	0.66	0.42	0.78	0.73	0.50

Fig. 8. Certain significantly different ($p < 0.05$) functions among the top 40 in relative abundance at KEGG pathway level 3 in natural symbiotic bacterial communities under different SMX concentrations.

other microalgal-bacterial consortia (Hu et al., 2022, Wang et al., 2022b), SMX significantly inhibited the growth of natural symbiotic bacteria associated with *Dunaliella* sp. (Fig. S2).

However, in complete contrast, the diversity of the natural symbiotic bacterial community significantly increased. Similar responses have been observed in bacterial communities under antibiotic stress in vegetable root systems (Ren et al., 2024) and vertical-flow constructed wetlands (Chen et al., 2020a). Recently, it was discovered for the first time that a symbiotic system of river-derived bacteria and *Chlorella vulgaris* exhibited increased diversity under SMX stress (Wang et al., 2024). Another distinction is that the enhanced connectivity in *Dunaliella* sp.'s natural symbiotic bacterial community under SMX stress indicates improved stability and adaptability to adverse conditions (Yuan et al., 2021).

The contrasting trends in diversity and connectivity between natural and foreign symbiotic bacterial communities may result from their distinct adaptation strategies. In foreign symbiotic consortia, host microalgae influence community composition more strongly than environmental factors through selectively recruiting specific bacteria (Kimbrel et al., 2019). Additionally, the decline in diversity is associated with the dominance of specific antibiotic-degrading bacteria (Xie et al., 2020), which are maintained through the accumulation and transfer of resistance genes (da da Silva Rodrigues et al., 2020).

In contrast, natural symbiotic consortia have stable species numbers, indicating weaker host selection pressure. From the perspective of specific species changes, *Labrenzia* has multiple pollutant resistance genes and exhibits strong aromatic compound degradation capabilities (Rodrigues Gisele et al., 2018). However, its abundance decreased, and no significant changes were observed in degradation-related pathways, suggesting that natural symbiotic bacteria may rely less on degradation strategies. Additionally, *Methylophaga* can promote nitrogen fixation and increase under nitrogen-limited conditions (Shi et al., 2018). However, it relies on phytoplankton for carbon and energy and competes for cobalamin (Bertrand et al., 2015). Its decline suggests poor adaptation to SMX stress, but it may also act as an adjustment strategy, creating growth opportunities for other bacteria. Notably, the abundance of *Ponticoccus* and *Mameliella* increased, and these bacteria are characterized by active quorum sensing (QS) functions or the presence of related genes (Danish-Daniel et al., 2016, Chi et al., 2017).

QS-mediated interactions is an important factor in shaping bacterial community structure and regulating ecological behaviors such as biofilm formation and antibiotic resistance (Zhou et al., 2016). Biofilm formation is an important bacterial adaptation strategy to environmental stresses because it can protect bacterial cells and enhance their resistance (Wu et al., 2015). The increase in QS function and biofilm formation phenotype in this study suggest that QS bacteria likely contribute to enhanced community connectivity by regulating biofilm formation. Additionally, the upregulation of amino acid metabolism in natural symbiotic bacteria reflects both active adaptation to SMX stress and a stronger interaction with microalgae, facilitated by increased EPS secretion (Wang et al., 2018). As a medium for metabolic exchange and signal communication, EPS serves as a critical link in enhancing the adaptability of *Dunaliella* sp. and its natural symbiotic bacteria to SMX stress.

4.4. Application prospects and challenges

In this study，most response trends generally follow a gradient pattern under different SMX concentration stresses, indicating that the symbiotic consortia can maintain stable adaptation mechanisms across a wide range of SMX levels. Although the symbiotic consortia show limited pollutant removal efficiency, its broad adaptability makes it a practical option for nutrient recovery in aquaculture wastewater with high antibiotic contamination risks. Its low adsorption and bioaccumulation characteristics also enhance its suitability as fishery feed, reducing source pollution risks while ensuring food safety. In antibiotic remediation, the biofilms from *Dunaliella* sp. symbiotic systems show great potential for integration with high-performance microalgae or degradative bacteria, which is conducive to enhancing the system's tolerance and stability. The response patterns of natural symbiotic bacteria provide guidance for foreign bacteria selection and community construction, which are beneficial for enhancing practical application potential.

Additionally, this study still has certain limitations: (1) The absence of a cell wall in *Dunaliella* sp. poses technical challenges for extracting bound EPS, limiting in-depth research on its role. (2) Considering the presence of symbiotic bacteria provides a more realistic understanding of microalgae's adaptation strategies, but understanding axenic microalgae's intrinsic traits is also crucial. However, removing symbiotic bacteria without stressing microalgae remains a major technical challenge. (3) Nutrient contents and microalgae growth determined the cultivation period. For practical applications, cost and efficiency factors require more efficient cultivation methods to improve feasibility. (4) Actual aquaculture wastewater may contain not only antibiotics but also risks of combined pollution from heavy metals and organic compounds, and environmental factors such as dissolved oxygen, pH, and salinity could also impact the symbiotic system. These are all important factors that cannot be overlooked in subsequent research and practical applications.

5. Conclusion

This study revealed that the *Dunaliella* sp. and its natural symbiotic bacterial consortium exhibited strong tolerance to SMX but weak removal efficiency. QS bacteria-driven community changes and biofilm formation enhance the diversity and connectivity of natural symbiotic bacterial communities. *Dunaliella* sp.'s high tolerance relies on strong antioxidant capacity (with CAT playing a key role) and elevated EPS secretion. EPS plays a key role in enhancing the adaptability of the consortium by strengthening the interaction between *Dunaliella* sp. and its natural symbiotic bacteria. However, the mechanisms regulating their interactions require further investigation. These findings strengthen our understanding of the responses of natural symbiotic bacteria to antibiotics, providing new insights for improving the adaptability of microalgae-bacteria symbiosis technology and the construction of bacterial communities.

CRediT authorship contribution statement

Zhao Hui: Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Pan Gang:** Writing – review & editing, Conceptualization. **Che Wenxue:** Visualization, Software, Methodology. **Tan Xiao:** Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Li Jinting:** Investigation. **Man Ying:** Methodology, Conceptualization. **Wang Liyi:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2025.104171.

Data availability

Data will be made available on request.

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