Interspecific competition between *Microcystis aeruginosa* and *Chlamydomonas microsphaera* stressed by tetracyclines

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Abstract
The extensive use of tetracyclines in human and veterinary medicine causes contamination in the environment that could contribute to the spread of antibiotic-resistant bacteria or competition between species of phytoplankton. In this study, *Microcystis aeruginosa* (a bloom-forming cyanobacterium) and *Chlamydomonas microsphaera* (common green alga) were selected to test the effects of different concentrations of tetracyclines (tetracycline and oxytetracycline) in monoculture and co-culture. The results showed that compared with monoculture, the cell growth of *C. microsphaera* decreased significantly in co-culture treated with different concentrations of tetracycline and oxytetracycline. The ratios of inhibition of *M. aeruginosa* exposed to 0.1, 2, and 10 mg L\(^{-1}\) of tetracycline varied between 17.7 and 31.37% in co-culture compared with monoculture, while the cell growth of *M. aeruginosa* was enhanced by treatment with 0.1, 2, and 7.25 mg L\(^{-1}\) of oxytetracycline in co-culture. However, the cell growth of *C. microsphaera* was significantly inhibited by all the treatments in co-culture. With the treatment of tetracycline, the specific growth rate of *M. aeruginosa* was 0.36 to 0.31 day\(^{-1}\) in monoculture and co-culture, while that of *C. microsphaera* ranged from 0.38 to 0.26 day\(^{-1}\) in monoculture, and it decreased from 0.25 day\(^{-1}\) (0 mg L\(^{-1}\)) to 0.08 day\(^{-1}\) (10 mg L\(^{-1}\)) in co-culture. With the treatment of oxytetracycline, the specific growth rate of *M. aeruginosa* was stimulated in co-culture, while that of *C. microsphaera* was significantly inhibited in co-culture compared with monoculture. Therefore, although *M. aeruginosa* significantly inhibited *C. microsphaera* in co-culture with the tetracycline-free treatment, the competitive advantage of *M. aeruginosa* expanded following the addition of low or high concentrations of tetracyclines.

Keywords Tetracyclines · Interspecific competition · *Microcystis aeruginosa* · *Chlamydomonas microsphaera* · Dominant species · Growth inhibition

Introduction
Phytoplankton, including microalgae and cyanobacteria, are primary producers and form the base of the food web in aquatic ecosystems (Seymour et al. 2017). Phytoplankton with small cell types are highly effective at taking up nutrients and converting light energy to chemical energy via photosynthesis (Häder et al. 2015). Since some species in the phytoplankton community are highly sensitive to environmental disturbance and pollution, some algal species have been used as environmental indicators for ecological assessments in terms of species composition, biomass, metabolism, and chemical byproducts (Pannard et al. 2009; Stevenson 2014; Bi et al. 2018).

*Microcystis*, the most common type of bloom-forming cyanobacteria, is widespread in eutrophic lakes and reservoirs, which causes environmental and ecological problems (Carmichael 1996). The release of metabolites, such as 2-methylisoborneol and geosmin, by cyanobacteria can cause odor problems (Sugimoto et al. 2016). It is also well-known that *Microcystis aeruginosa* produces the hepatotoxin microcystin that deteriorates water quality and threatens the health of livestock and humans (Watanabe et al. 1988; Hee Jin et al. 2005; Zhai et al. 2013). *Chlamydomonas*
microsphaera is a common and nontoxic single-celled green alga in freshwater ecosystems (Song et al. 2007; Zhang et al. 2012). M. aeruginosa and C. microsphaera normally coexist in the same aquatic system and dominate along with spatial and temporal variations, respectively (Zhang et al. 2013). The phytoplankton community can be affected by abiotic and biotic factors, including pH, light, nutrients, temperature, and allelopathic interactions (Zhang et al. 2012; Sugimoto et al. 2016; Tan et al. 2019b, 2020). Many studies have described how external contaminants can also change the community structure. A low concentration of pentachlorophenol (1 μg L⁻¹) that was close to the concentration in surface water stimulated the growth of M. aeruginosa and caused a shift in dominance from M. aeruginosa to Chlorella vulgaris when the concentration of pentachlorophenol > 0.25 mg L⁻¹ (de Morais et al. 2014). M. aeruginosa outcompeted Scenedesmus obliquus in co-culture without linear alkylbenzene sulfonate (LAS) or 1 mg L⁻¹ of LAS, while S. obliquus dominated when the concentrations of LAS > 20 mg L⁻¹ (Zhu et al. 2016). Similarly, Tan et al. (2019a) investigated the effect of phenol on competition between M. aeruginosa and Chlorella pyrenoidosa in co-culture, and described that phenolic pollution overturned the competition between both species.

Tetracyclines are one of the most extensively used classes of antibiotics in agricultural activities, livestock, and aquaculture because of their broad-spectrum activity, cost efficiency, more effective antimicrobial activity, and low toxicity. Tetracyclines found in Chinese river or lake basins ranged from 67 to 25,538 ng L⁻¹ for tetracycline, 170 to 361,107 ng L⁻¹ for oxytetracycline, and 267 to 25,538 ng L⁻¹ for chlortetracycline (Jiang et al. 2014; Wang et al. 2017a, b). However, high concentrations (100 to 500 mg L⁻¹) of antibiotics were found in pharmaceutical manufacturing wastewater (Jing et al. 2014), which require further treatment. The solution for waste antibiotics is the subject of intensive research, and the wastewater treatment for tetracyclines can be categorized as biochemical and physicochemical technologies. Biochemical processes primarily involve biodegradation by activated sludge, while physicochemical technologies include adsorption, oxidation, membrane processes, photocatalysis, and electrochemical methods (Jing et al. 2014; Scaria et al. 2021). However, owing to the toxicity of antibiotics toward activated sludge and problems with the application of techniques, the removal of tetracyclines was found to be incomplete, and up to 32 mg L⁻¹ of tetracyclines were detected in the effluent of a pharmaceutical wastewater treatment plant (Hou et al. 2016).

The effect of oxytetracycline and sulfamethoxazole on the physiological characteristics of M. aeruginosa and C. microsphaera was studied individually (Zhou et al. 2021). In this study, the effect of tetracyclines on the interspecific competition between the toxic Microcystis strain (M. aeruginosa) and the nontoxic Chlamydomonas strain (C. microsphaera) was studied. These effects were analyzed by the growth of cells in monoculture and co-culture and by established competitive parameters. This study aimed to evaluate the dominant species on the phytoplankton community when grown in the absence of tetracyclines or exposed to them.

Materials and methods

Organisms and reagents

M. aeruginosa (FACHB-1343) and C. microsphaera (FACHB-52) were purchased from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). Tetracycline and oxytetracycline (Aladdin, Shanghai, China) were selected as test pharmaceuticals.

The media, including WC (Guillard and Lorenzen 1972), BG11, SE, and SP, were pre-selected before the study of the effect of antibiotics on algae competition. The composition of the media is shown in Tables S1 and S2. Two algae were cultured in different media, and the medium (BG-11) in which the algae grew best was selected for their culture. M. aeruginosa and C. microsphaera were cultured separately in BG11 medium for 1 week before the experiment was initiated.

Experimental design

The monoculture and co-culture were designed to identify the interspecific competition between M. aeruginosa and C. microsphaera. The amount of inoculation was based on the total volume ratio of M. aeruginosa and C. microsphaera at 1:1. Thus, the initially inoculated concentration of algae cells of M. aeruginosa and C. microsphaera were 10⁶ and 10⁴ cells mL⁻¹ in monoculture and co-culture, respectively. The two species were cultured under axenic conditions in 250-mL Erlenmeyer flasks that contained 150 mL of the medium at 25 ± 1 °C with a light:dark cycle of 12:12 h under illumination by a cool-white fluorescent light at 50-μmol photons m⁻² s⁻¹. Monocultures and co-cultures were treated with four treatment groups of tetracycline (0, 0.1, 2, and 10 mg L⁻¹) and oxytetracycline (0, 0.1, 2, and 7.25 mg L⁻¹). The range of concentration of the tetracyclines was based on the 96-h half-maximal inhibitory concentration (IC₅₀) of both species as shown in Table S3, and the range of concentration of oxytetracycline was established based on Zhou et al. (2021). Each treatment was performed in triplicate, and the flasks were shaken twice every day and placed randomly.
Determination of phytoplankton biomass

The cell densities of both organisms were quantified in monoculture using a spectrophotometer (UV-1600PC; Shanghai Mapade Instruments Co., Ltd., Shanghai, China) and counted with a hemocytometer and a microscope (XSP-2CA; Shanghai Optical Instrument Factory, Shanghai, China). The linear regression between the cell density and optical density at 675 nm had a high correlation index ($R^2 > 0.99$) as shown in Fig. S1. The cell densities of both species in co-culture were counted directly with the hemocytometer and the microscope. The cells were counted on days 1, 3, 5, 7, 9, 11, 13, and 15.

Statistical analysis

The parameters of interspecific competition determined for the significant differences between monoculture and co-culture were analyzed by a Student $t$-test. The significant differences in the means of the variables between different concentrations of tetracyclines were analyzed by a one-way analysis of variance (ANOVA) using the LSD test ($P < 0.05$). All the results were expressed as the mean ± standard deviation. The IC$_{50}$ values were analyzed using SPSS 23.0 (IBM, Inc., Chicago, IL, USA).

The effects of co-culture on the growth of algae can be described by the ratio of cell inhibition described as follows:

\[
\frac{N_{C+1} - N_C}{t_{n+1} - t_n} = r_C N_C (1 - \frac{N_C + \alpha N_M}{K_C})
\]

where $N_{C+1}$ ($N_C$) and $N_{M+1}$ ($N_M$) represent the cell density of $C$. microsphaera and $M$. aeruginosa under co-culture at day $t_{n+1}$ ($t_n$). $K_C$ and $K_M$ represent the maximum cell density of $C$. microsphaera and $M$. aeruginosa in monoculture, respectively. $r_C$ and $r_M$ represent the intrinsic cell growth rate of $C$. microsphaera and $M$. aeruginosa, respectively. $\alpha$ (the effect of $M$. aeruginosa on $C$. microsphaera) and $\beta$ (the effect of $C$. microsphaera on $M$. aeruginosa) are the competition coefficients under co-culture.

Results

Growth of $M$. aeruginosa and $C$. microsphaera treated with or without tetracycline in monoculture and co-culture

The growth curves of $M$. aeruginosa and $C$. microsphaera in monoculture and co-culture treated with different concentrations of tetracycline for 15 days are shown in Fig. 1. In monoculture, compared with the tetracycline-free treatment, the same growth trend of $M$. aeruginosa was observed following exposure to low concentrations of tetracycline (0.1 and 2 mg L$^{-1}$), and the concentrations of $M$. aeruginosa cells were enhanced during the culture time when treated with 0.1 and 2 mg L$^{-1}$ of tetracycline. However, the growth of $M$. aeruginosa was inhibited with 10 mg L$^{-1}$ of tetracycline. The growth of $C$. microsphaera was simulated at 0.1 mg L$^{-1}$ of tetracycline, but it was significantly inhibited at 2 and 10 mg L$^{-1}$ of tetracycline in monoculture.

In co-culture, the cell growth of both species was accelerated at 0.1 mg L$^{-1}$ of tetracycline but inhibited by 2 and 10 mg L$^{-1}$ of tetracycline compared with the tetracycline-free treatment. The biomass of $C$. microsphaera decreased significantly in co-culture treated with different concentrations of tetracycline compared with the monoculture. In the absence of tetracycline, after 15 days of culture, the cell concentration in monoculture of $C$. microsphaera was 13.1-fold higher than that in co-culture.

Figure 2 shows the cell growth of $M$. aeruginosa and $C$. microsphaera treated with different concentrations of oxytetracycline for 15 days. In monoculture, the cell growth of $M$. aeruginosa and $C$. microsphaera increased similarly between the treatment of low concentration of oxytetracycline (0.1 mg L$^{-1}$) and the oxytetracycline-free treatment. Compared with the oxytetracycline-free treatment, the degree of inhibition of the biomass of $C$. microsphaera (52.9%) was higher than that of $M$. aeruginosa (13.7%) when
treated with 2 mg L\(^{-1}\) of oxytetracycline. Both species were significantly inhibited with 7.25 mg L\(^{-1}\) of oxytetracycline. In co-culture, the cell growth of *M. aeruginosa* was enhanced at 0.1 and 2 mg L\(^{-1}\) compared with the oxytetracycline-free treatment, and after 15 days of culture, the biomass of *M. aeruginosa* treated with oxytetracycline at 0.1 and 2 mg L\(^{-1}\) increased by 68.5% and 24.2%, respectively. The degree of inhibition of the biomass of *M. aeruginosa* treated with 7.25 mg L\(^{-1}\) of oxytetracycline in co-culture was lower than that in monoculture. In contrast to monoculture, the growth of *C. microsphaera* cells treated with different concentrations of oxytetracycline was markedly inhibited in co-culture.

**Growth parameters**

The growth kinetic parameters of *M. aeruginosa* and *C. microsphaera* in both cultures treated with tetracycline
and oxytetracycline were calculated using Eq. (3) and are shown in Tables 1 and 2, respectively. A high coefficient of regression ($R^2$) indicates that the cell growth of *M. aeruginosa* and *C. microsphaera* in Figs. 1 and 2 was well-fitted by the logistic growth model. The maximum cell density ($K$) of *M. aeruginosa* and *C. microsphaera* treated with tetracycline in monoculture was higher than that in co-culture (Table 1), which indicates that the cell growth of *M. aeruginosa* and *C. microsphaera* was limited in co-culture compared with that in monoculture. In monoculture, a comparison of different concentrations of tetracycline treatment indicated that the maximum cell density of *M. aeruginosa* was higher at 0.1 and 2 mg L$^{-1}$ of tetracycline than that of the tetracycline-free treatment, while the maximum cell density of *M. aeruginosa* in co-culture was the highest in tetracycline-free group. The maximum cell density of *C. microsphaera* was the highest with

### Table 1

The growth kinetic parameters of *Microcystis aeruginosa* and *Chlamydomonas microsphaera* in monoculture and co-culture treated with tetracycline

| Species         | Culture type | Tetracycline (mg L$^{-1}$) | $K$ (10$^6$ cells mL$^{-1}$) | $a$ | $r$ | $R^2$ |
|-----------------|--------------|----------------------------|------------------------------|-----|-----|-------|
| *M. aeruginosa* | Monoculture  | 0                          | 497.8                        | 5.04| 0.32| 0.998 |
|                 |              | 0.1                        | 565.0                        | 5.17| 0.31| 0.998 |
|                 |              | 2                          | 456.6                        | 4.39| 0.28| 0.998 |
|                 |              | 10                         | 185.2                        | 4.82| 0.38| 0.983 |
|                 | Co-culture   | 0                          | 346.2                        | 3.78| 0.27| 0.984 |
|                 |              | 0.1                        | 220.4                        | 3.78| 0.35| 0.979 |
|                 |              | 2                          | 185.6                        | 3.51| 0.32| 0.971 |
|                 |              | 10                         | 145.0                        | 3.99| 0.34| 0.983 |
| *C. microsphaera* | Monoculture  | 0                          | 4.58                         | 4.09| 0.35| 0.995 |
|                 |              | 0.1                        | 4.69                         | 3.88| 0.35| 0.996 |
|                 |              | 2                          | 3.11                         | 4.13| 0.33| 0.998 |
|                 |              | 10                         | N.A                          | 11.61| 0.30| 0.963 |
|                 | Co-culture   | 0                          | 0.28                         | 2.59| 0.41| 0.956 |
|                 |              | 0.1                        | 0.38                         | 2.60| 0.34| 0.987 |
|                 |              | 2                          | 0.22                         | 2.28| 0.33| 0.960 |
|                 |              | 10                         | 0.04                         | 0.87| 0.77| 0.559 |

$K$, maximum cell density; N.A., data not available; $r$, intrinsic cell growth in monoculture.

### Table 2

The growth kinetic parameters of *Microcystis aeruginosa* and *Chlamydomonas microsphaera* in monoculture and co-culture treated with oxytetracycline

| Species         | Culture type | Oxytetracycline (mg L$^{-1}$) | $K$ (10$^6$ cells mL$^{-1}$) | $a$ | $r$ | $R^2$ |
|-----------------|--------------|-------------------------------|------------------------------|-----|-----|-------|
| *M. aeruginosa* | Monoculture  | 0                            | 339.5                        | 3.99| 0.34| 0.996 |
|                 |              | 0.1                          | 367.1                        | 4.37| 0.35| 0.996 |
|                 |              | 2                            | 322.1                        | 4.44| 0.35| 0.998 |
|                 |              | 7.25                         | 243.1                        | 4.67| 0.09| 0.496 |
|                 | Co-culture   | 0                            | 346.2                        | 3.78| 0.27| 0.984 |
|                 |              | 0.1                          | 343.8                        | 4.21| 0.45| 0.976 |
|                 |              | 2                            | 327.6                        | 3.82| 0.33| 0.986 |
|                 |              | 7.25                         | 130.5                        | 6.13| 0.56| 0.995 |
| *C. microsphaera* | Monoculture  | 0                            | 1957.5                       | 10.59| 0.28| 0.989 |
|                 |              | 0.1                          | 2096.3                       | 10.59| 0.27| 0.986 |
|                 |              | 2                            | 1054.6                       | 11.31| 0.32| 0.993 |
|                 |              | 7.25                         | 0.73                         | 4.15| 0.25| 0.990 |
|                 | Co-culture   | 0                            | 0.55                         | 3.38| 0.60| 0.995 |
|                 |              | 0.1                          | 0.50                         | 3.57| 0.65| 0.970 |
|                 |              | 2                            | 0.34                         | 3.08| 0.46| 0.963 |
|                 |              | 7.25                         | 0.16                         | 2.32| 0.40| 0.928 |

$K$, maximum cell density; $r$, intrinsic cell growth in monoculture.
treatment of 0.1 mg L\(^{-1}\) of tetracycline treatment in both monoculture and co-culture.

The \(K\) of \(M.\ aeruginosa\) and \(C.\ microsphaera\) treated with oxytetracycline in monoculture was also higher than that in co-culture (Table 2). The maximum cell density of \(M.\ aeruginosa\) was the highest at 0.1 mg L\(^{-1}\) of tetracycline treatment in monoculture and with tetracycline-free treatment in co-culture. However, in monoculture, under 0, 0.1, and 2 mg L\(^{-1}\) of oxytetracycline treatment, the calculated maximum cell density of \(C.\ microsphaera\) was too high, which indicated that the cells were in the exponential growth phase for 15 days. This suggests that the culture time should be extended to calculate the maximum cell density. Similarly, in cases where the cell growth curve did not reach a plateau (treatment with tetracycline and oxytetracycline at 0, 0.1, or 0.2 mg L\(^{-1}\) in both cultures), the value of \(K\) predicted by the model could be higher than the actual value.

Figure 3 describes the effect of co-culture on the ratios of cell inhibition of \(M.\ aeruginosa\) and \(C.\ microsphaera\) treated with different concentrations of tetracycline at day 15. \(M.\ aeruginosa\) and \(C.\ microsphaera\) were inhibited in co-culture treated with different concentrations of tetracycline. The ratios of inhibition of \(M.\ aeruginosa\) varied between 8.2 and 31.4%, while the ratios of inhibition of \(C.\ microsphaera\) were approximately 90.2 to 93.5%. In terms of feeding oxytetracycline, the ratios of inhibition of \(M.\ aeruginosa\) were 22.2% in the oxytetracycline-free group and all negative values in the oxytetracycline-treated groups, which indicated that the growth of \(M.\ aeruginosa\) cells treated with oxytetracycline was enhanced. The ratios of inhibition of \(C.\ microsphaera\) decreased from 88.0% (0.1 mg L\(^{-1}\) of oxytetracycline) to 25.6% (7.25 mg L\(^{-1}\) of oxytetracycline).

The specific growth rates (\(\mu\)) of \(M.\ aeruginosa\) and \(C.\ microsphaera\) treated with tetracycline are shown in Fig. 4. The specific growth rates of \(M.\ aeruginosa\) were from 0.36 to 0.31 day\(^{-1}\) in monoculture and co-culture following exposure to tetracycline. In monoculture, the specific growth rate of \(M.\ aeruginosa\) decreased significantly at 10 mg L\(^{-1}\) of tetracycline, while the rates of \(M.\ aeruginosa\) at 2 and 10 mg L\(^{-1}\) of tetracycline were significantly different from the rates at 0 and 0.1 mg L\(^{-1}\) of tetracycline in co-culture. Significant differences were found between the growth of monoculture and co-culture in the specific growth rate of \(M.\ aeruginosa\) following exposure to all concentrations of tetracycline. The specific growth rates of \(C.\ microsphaera\) decreased from 0.38 day\(^{-1}\) at 0.1 mg L\(^{-1}\) of tetracycline to 0.26 day\(^{-1}\) at 10 mg L\(^{-1}\) of tetracycline in monoculture, while the rates decreased from 0.25 day\(^{-1}\) (0 mg L\(^{-1}\)) to 0.08 day\(^{-1}\) (10 mg L\(^{-1}\)) in co-culture. Highly significant differences (\(P<0.01\)) were identified between monoculture and co-culture in the tetracycline-free and tetracycline-treated groups.

In monoculture, the specific growth rate of \(M.\ aeruginosa\) decreased significantly at 10 mg L\(^{-1}\) of oxytetracycline (Fig. 5). In co-culture, the highest specific growth rate of \(M.\ aeruginosa\) was 0.39 day\(^{-1}\) at 0.1 mg L\(^{-1}\) of oxytetracycline. The specific growth rates of \(M.\ aeruginosa\) in co-culture were higher than those in monoculture. Significant differences between the two types of culture were observed at 0.1 and 7.25 mg L\(^{-1}\) of oxytetracycline. In terms of \(C.\ microsphaera\), the specific growth rates decreased from 0.37 day\(^{-1}\) in oxytetracycline-free treatment to 0.21 day\(^{-1}\) at 10 mg L\(^{-1}\) of oxytetracycline.
The increase in oxytetracycline concentration. The value of microsphaera decreased with the increase in oxytetracycline concentration. This indicated that Microcystis aeruginosa barely affects the growth of Chlamydomonas microsphaera in monoculture. All the negative values of microsphaera α, effect of M. aeruginosa on C. microsphaera; β, effect of C. microsphaera on M. aeruginosa.

Table 3 lists the competition parameters α (the effect of M. aeruginosa on C. microsphaera) and β (the effect of C. microsphaera on M. aeruginosa). α < 0.1 following treatment with 0, 0.1, and 2 mg L⁻¹ of tetracycline, while α increased to 27.8 with 10 mg L⁻¹ of tetracycline. This suggests that M. aeruginosa barely affects the growth of C. microsphaera when tetracycline < 2 mg L⁻¹. However, M. aeruginosa inhibited the growth of C. microsphaera at 10 mg L⁻¹ of tetracycline. All the negative values of β in all the treatments of tetracycline suggest that C. microsphaera enhanced the growth of M. aeruginosa. When oxytetracycline was added, α decreased with the increase in oxytetracycline concentration. This indicated that M. aeruginosa inhibited the growth of C. microsphaera, and the degree of inhibition decreased with the increase in oxytetracycline concentration. The value of β was also negative in all the treatments with oxytetracycline, indicating that C. microsphaera enhanced the growth of M. aeruginosa.

| Tetracyclines | mg L⁻¹ | α       | β            |
|--------------|--------|---------|--------------|
|              |        |         |              |
| Tetracycline | 0      | 0.04 ± 0.02 | −1181 ± 22  |
|              | 0.1    | 0.04 ± 0.02 | −1156 ± 1657|
|              | 2      | 0.03 ± 0.02 | −578 ± 433  |
|              | 10     | 27.80 ± 5.82 | −569 ± 293  |
| Oxytetracycline | 0    | 17.03 ± 2.71 | −282 ± 55   |
|              | 0.1    | 9.53 ± 3.67  | −552 ± 325  |
|              | 2      | 6.86 ± 2.77  | −230 ± 19   |
|              | 7.25   | 0.01 ± 0.02  | −16.607 ± 4795|

α, effect of M. aeruginosa on C. microsphaera; β, effect of C. microsphaera on M. aeruginosa.
Discussion

Interspecific competition in tetracycline-free treatment

The effect of co-culture on the cell growth of both species indicated the ratio of inhibition of the cell growth of Chlamydomonas microsphaera (approximately 90%) was higher than that of M. aeruginosa (<23%) in the absence of tetracyclines. Moreover, compared with monoculture, the specific growth rate of M. aeruginosa was not affected in co-culture, but the rate of C. microsphaera decreased significantly in co-culture. According to the competitive coefficient of the Lotka-Volterra model, the effect of M. aeruginosa on C. microsphaera (α) with the tetracycline-free treatment was positive, indicating that M. aeruginosa inhibited the growth of C. microsphaera. The negative values of β indicated that C. microsphaera enhanced the growth of M. aeruginosa. Thus, these parameters confirmed the dominance of M. aeruginosa in co-culture. The same phenomenon has been previously described. M. aeruginosa became the dominant species when competing with Scenedesmus in co-culture (Zhu et al. 2016).

The dominance of M. aeruginosa could be due to the size of its cells. Microcystis is 6- to 12-fold smaller than Scenedesmus in cell volume (Zhu et al. 2016). C. microsphaera is approximately 100-fold larger than M. aeruginosa according to the results of this study. The cell size of phytoplankton affects the rates of nutrient uptake, as well as nutrient diffusion (Finkel et al. 2010), while the smaller size and large surface-to-volume ratio allow a high competitive capacity of absorbing nutrients (Azam et al. 1983; Friebele et al. 1978). This could explain the competitive advantage of M. aeruginosa for nutrients compared with C. microsphaera. Furthermore, the microcysts and anatoxin produced by M. aeruginosa could affect the dominant species. Microcystins (such as microcystin-LR) and anatoxin-a, are products of M. aeruginosa that influence the growth and physiology of eukaryotic and prokaryotic phytoplankton (Chia et al. 2019). Microcystins not only reduce the survival and growth rate of fish by interfering with embryonic hatching but also trigger histopathological effects (Malbrouck and Kestemont 2006).

It is interesting to note that M. aeruginosa was stimulated to increase its production of microcysts in the presence of green algae (Bittencourt-Oliveira et al. 2015). Although the total biomass of zooplankton was not affected by exposure to microcystin, this compound had a positive effect at the population level (Paes et al. 2016). In addition, extracellular allelopathic compounds, such as D-limonene and 1-chlorine heptacosane, produced by M. aeruginosa could contribute to its dominance (Zhai et al. 2013). The negative allelopathic effects of M. aeruginosa exudates significantly inhibited the growth of two common green algae (Scenedesmus quadricauda and Chlorella pyrenoidosa) and a diatom (Cyclotella meneghiniana) in both the exponential and stationary phases (Wang et al. 2017a, b). The combined efforts of the nutrient competition and the negative allelopathic effect of the cyanobacteria could explain why the specific growth rate of C. microsphaera in co-culture was significantly lower than that in monoculture in the absence of tetracycline treatments in our work.

Interspecific competition under different concentration of tetracyclines

Hormesis is a dose–response relationship characterized by low-dose stimulation and high-dose inhibition (Stebbing 1982; Axelrod et al. 2004). The hormesis of pollutants has been reported previously. Tan et al. (2019a, b) found that phenol stimulated the growth of M. aeruginosa at low concentrations (2 mg L⁻¹). The growth of Microcystis aeruginosa was accelerated at florfenicol concentration < 2 mg L⁻¹ and significantly inhibited > 4 mg L⁻¹ (Liu et al. 2012). The growth hormesis of M. aeruginosa was also observed in this study with the exposure of low concentrations of tetracycline and oxytetracycline. The cell growth of M. aeruginosa was enhanced with low concentrations of tetracyclines (0.1 or 2 mg L⁻¹) in monoculture or co-culture and inhibited with high concentrations of tetracyclines.

The cell response of hormesis and allelopathy could co-stimulate the change in community structure, which was demonstrated from the competition parameters. The Lotka-Volterra model is used as an extension of the logistic equation to depict the dynamics of ecological systems with two competing species and to quantify interspecific competition between two species (Shim and Fishwick 2008; Mallet 2012). The Lotka-Volterra competition model has been widely applied in systems that include animals, plants, and microorganisms (Inouye 2001; Song et al. 2014). Many studies have also applied this model to phytoplankton interspecific competition. In addition, the model can be further extended to study the competition between two species in different cultural conditions. For example, He et al. (2016) addressed interspecific competition between algal species under different temperatures (15, 20, and 25 °C) and lighting conditions (40, 60, and 80 μmol photons m⁻² s⁻¹). Tan et al. (2020) calculated the competitive parameters between green algae and cyanobacteria under different wavelengths of light (white 400–700 nm, red 620–700 nm, and blue 410–490 nm). Interspecific competition for nutrients, such as nitrogen and phosphorus, between macrophytes and green algae was also investigated (Tan et al. 2019b; Zhang et al. 2019). The application of this model has also been extended to study the competition between phytoplankton species.
under the stress of pollutants, such as anthracene (Bi et al. 2015), LAS (Zhu et al. 2016), phenol (Tan et al. 2019a), and the polycyclic aromatic hydrocarbon phenanthrene (Jin et al. 2014). In this study, the Lotka-Volterra model was used to identify the interspecific competition between cyanobacteria and green algae under the stress of tetracyclines. The positive values of $a$ (the effect of *M. aeruginosa* on *C. microsphaera*) and the negative values of $\beta$ (the effect of *C. microsphaera* on *M. aeruginosa*) in the tetracycline treatments demonstrated that *M. aeruginosa* inhibited the growth of *C. microsphaera*, while *C. microsphaera* promoted the growth of *M. aeruginosa*. This phenomenon of inhibition or promotion was manifested by the ratio of inhibition of these two species. The ratio of inhibition indicated that the addition of tetracyclines significantly inhibited the growth of *C. microsphaera*, while the inhibition of *M. aeruginosa* was much less pronounced. In fact, its growth could even be stimulated.

In monoculture, the specific growth rates of *M. aeruginosa* and *C. microsphaera* were not affected under the stress of low concentrations of tetracyclines, while the specific growth rate of *M. aeruginosa* was inhibited to a lower degree than that of *C. microsphaera* following exposure to high concentrations of tetracyclines. In co-culture, this situation was exacerbated, i.e., the specific growth rate of *M. aeruginosa* was less affected or even promoted under the exposure of tetracyclines, but the specific growth rate of *C. microsphaera* was significantly inhibited following exposure to low concentrations of tetracyclines. In contrast, following exposure to a high concentration of tetracyclines, the specific growth rate of *C. microsphaera* was inhibited to a higher degree than that in monoculture. The different responses of varying species to pollutants could be one reason for this phenomenon. *M. aeruginosa* and *C. microsphaera* were tested for acute toxicity from tetracyclines, and a 96-h IC$_{50}$ was identified. Table S3 shows that the IC$_{50}$ of tetracycline for *M. aeruginosa* and *C. microsphaera* was 10.39 and 2.04 mg L$^{-1}$, respectively, whereas the IC$_{50}$ of oxytetracycline was 7.25 and 4.20 mg L$^{-1}$, respectively (Zhou et al. 2021). This indicated that *M. aeruginosa* has stronger antibiotic resistance than *C. microsphaera*. Thus, low concentrations of tetracyclines stimulated the growth of *M. aeruginosa* and inhibited the growth of *C. microsphaera*, while high concentrations of tetracyclines more strongly inhibited the growth of *C. microsphaera* compared with that of *M. aeruginosa*, which promoted the dominance of *M. aeruginosa* in the presence of tetracyclines. Furthermore, as described in the tetracycline-free treatment, the combined efforts of the nutrient competition and the negative allelopathic effect of the cyanobacteria also inhibited the growth rate of *C. microsphaera*, while the addition of tetracyclines exacerbated the reduction in the growth rate of *C. microsphaera*.

Moreover, some studies have described how pollutants can overturn the species community structure. Zhu et al. (2016) described how the dominant species changed from *M. aeruginosa* to *S. obliquus* when LAS $> 20$ mg L$^{-1}$ in co-culture. *M. aeruginosa* dominated following exposure to 0, 2, and 20 mg L$^{-1}$ of phenol in co-culture, while *Chlorella pyrenoidosa* outcompeted with 200 mg L$^{-1}$ of phenol (Tan et al. 2019a). However, in this study, the dominance of *M. aeruginosa* with all the treatments of tetracyclines differed from previous studies. This can be explained by the resistance to toxicity. The IC$_{50}$ of LAS for *M. aeruginosa* and *S. obliquus* was 10 and 100 mg L$^{-1}$, respectively. In terms of phenol treatments, the IC$_{50}$ for *M. aeruginosa* and *C. pyrenoidosa* was 80.8 and 631.4 mg L$^{-1}$, respectively. *M. aeruginosa* was more sensitive than *S. obliquus* and *Chlorella pyrenoidosa*. When the concentration of pollutants exceeds the range of tolerance of the cells, the cell growth is affected, which leads to changes in the community structure. In this study, *M. aeruginosa* was more tolerant than *C. microsphaera*. Thus, following treatment with high concentrations of tetracyclines, *M. aeruginosa* dominated in all the treatments. Therefore, it could be concluded that the community structure of organisms during co-culture is also affected by the toxicity of the pollutants on different concentrations.

Collectively, in the presence of tetracyclines, *M. aeruginosa* dominated in co-culture with *C. microsphaera*. *Microcystis* has been reported to be more competitive in co-culture under eutrophic conditions compared with *S. obliquus* (Zhu et al. 2016). *Chlorella pyrenoidosa* (green algae) (Tan et al. 2019a), and *Monoraphidium convolutum* (green algae) (Bittencourt-Oliveira et al. 2015). However, *Cylindrospermopsis raciborskii* (Cyanobacteria) outcompeted *M. aeruginosa* with a high concentration of phosphorus. The interspecific competition is dependent on a combination of factors that relate to the species or strain communities or variable environments, such as light, temperature, or nutrients (Sugimoto et al. 2016; Xiao et al. 2017). The presence of more complex interactions and abiotic conditions, particularly the different possible trophic status of the systems, indicates that the conclusions from this study (the competitive advantage of *M. aeruginosa* under the stress of antibiotics) do not extend to the real environment in a general way. In addition, it is expected to find better competitors and more resistant species than *M. aeruginosa* in natural phytoplankton communities. Although the tetracyclines do not reach the level of mg L$^{-1}$ in the natural river and lake basins, the stimulation by low concentrations of antibiotics, such as oxytetracycline, also needs to be considered in interspecific competition.
Conclusions

This study investigated the interspecific competition between cyanobacteria (M. aeruginosa) and green algae (C. microsphaera) in the absence and presence of tetracycline and oxytetracycline. The results of our study demonstrate that M. aeruginosa is a superior competitor to C. microsphaera in the co-culture system. In the tetracycline-free treatment, M. aeruginosa significantly inhibited the growth of C. microsphaera in co-culture. C. microsphaera was more sensitive to tetracyclines compared with M. aeruginosa. In co-culture, low concentrations of tetracyclines had no effect or stimulated the growth of M. aeruginosa but had no effect or inhibited the growth of C. microsphaera. High concentrations of tetracyclines more strongly inhibited the rate of growth of C. microsphaera than that of M. aeruginosa, which enhanced the competitive advantage of M. aeruginosa and intensified the dominance of M. aeruginosa in the presence of tetracyclines.

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Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by XZ and XJ. The first draft of the manuscript was written by XZ and reviewed by JC and PG. All the authors commented on the previous versions of the manuscript. All the authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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