Bacterial abundance and community structure in response to nutrients and photodegraded terrestrial humic acids in a eutrophic lake

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Abstract

The exposure of humic substances to solar radiation can alter their concentration and composition and subsequently influences their bioavailability in aquatic food webs. With eutrophication increasingly prominent in lakes, nutrients, such as inorganic N and P, are a prerequisite for heterotrophic bacteria that use organic matter. Here photodegradation of terrestrial humic acids and nutrient addition were performed to investigate the response of bacterial abundance and community structure to photodegraded humic acids and increased nutrient concentrations in a eutrophic lake. Results showed that the decreasing level of absorption coefficient at 460 nm in the treatment irradiated with 40 W UV lamps was more remarkable than that of the treatment irradiated with 20 W UV lamps and the control. This reduced coefficient corresponds to the greatest decrease in humic acid concentration in the 40 W group. Bacteria showed high abundance after incubation with humic acids which underwent strong irradiation intensity. An increased nutrient concentration significantly affected bacterial abundance. The dominant bacteria were *Aquabacterium* for the irradiated group, *Aquabacterium* and *Limnobacter* for the 20 W group and *Flavobacterium* and *Limnobacter* for the 40 W group. *Armatimonadetes-gp4* and *Sediminibacterium* showed evident response to high nutrient concentration. Our results showed that the exposure of terrestrial humic acids to UV light and the increasing concentration of nutrients have obviously changed bacterial community.

Keywords Soil dissolved organic matter · Absorption coefficient · Bacterial abundance · Bacterial diversity

Introduction

Terrestrial dissolved organic matter (TDOM) is mainly derived from the tissues of terrestrial plants, which are subsequently modified in the soil and delivered to aquatic ecosystems (Solomon et al. 2015). Some evidence suggests that terrestrial organic matter plays a key role in carbon balance and ecosystem dynamics (Jansson et al. 1999; Cole et al. 2006; Cole et al. 2011). TDOM can greatly contribute to aquatic bacterial production (Moran and Hodson 1994; Cole et al.

2002; Zhou et al. 2021), as evidenced by findings that respiration exceeds gross primary production in net heterotrophic aquatic systems (Kritzberg et al. 2004; Karlsson et al. 2012). Moreover, the dominant microorganisms in oligotrophic lakes with low chlorophyll *a* concentration of 2–5 μg/L are heterotrophic bacteria rather than phytoplanktons (del Giorgio and Gasol 1995). The subsidy of consumer metabolism by TDOM is not only in oligotrophic and/or humic lakes, but also in eutrophic lakes (Carpenter et al. 2005; Cole et al. 2011; Su et al. 2017). Thus, the interaction between TDOM and bacterial community has a great influence in aquatic carbon balance and microbial ecology.

A large amount of TDOM and inorganic nutrients, e.g. nitrogen and phosphorus, are delivered to lakes during the process of deforestation, urbanisation and anthropogenic discharges (Zhou et al. 2021). Humic substances, as representative components of TDOM, are generally bio-refractory due to their complex molecular structure (McDonald et al. 2004; Guillemette et al. 2016). The bioavailability of humic substances is related to its biochemical composition and

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environmental factors, such as nutrient and ultraviolet (UV) radiation (Amon and Benner 1996; Vähätalo and Järvinen 2007).

Humic substances can absorb UV and visible radiation; the exposure of humic substances to solar radiation alters their concentration and composition, subsequently influencing their bioavailability (Vähätalo and Wetzel 2004; Boyle et al. 2009; Miranda et al. 2020). A positive function of irradiated soil humic substances on the ability of DOM to promote bacterial growth has been found (Anesio et al. 2005; Tranvik and Bertilsson 2001). Chromophoric dissolved organic matter (CDOM)-absorbed photons were highly important for the transformation of organic materials in lakes at boreal Sweden and arctic latitudes having high CDOM concentrations (Koehler et al. 2014; Groeneveld et al. 2015; Koehler et al. 2018). In humic lakes, terrestrial carbon accounts for the major contribution of bacterial carbon contents and microbial energy flow (Tranvik and Bertilsson 2001; Kritzberg et al. 2006). It was found that the UV absorbance value (SUVA254) was significantly negatively correlated with the abundance of plankton, indicating that humic substances in lakes and rivers could promote the growth of bacteria (Ye et al. 2014). This finding is probably because terrestrial humic matter derived from vascular and ligninaceous plants can be photodegraded to substrates that support bacterial growth (Tranvik and Bertilsson 2001). Microorganisms, particularly bacteria, are the main driving factors in lake biogeochemical cycles. However, it remains a challenge to study the composition of bacteria community structure under environmental changes. Eutrophication has become an extensively environmental problem for lakes in the past four decades (Carpenter et al. 1999; Ho et al. 2019). It is the consequence of high nutrient loading and is closely related to human activities (Pinturier-Geiss et al. 2002; Hu et al. 2008). Nutrients, such as inorganic N and P, are a prerequisite for heterotrophic bacteria that use organic matter. Phytoplankton normally competes with bacteria for nutrients in oligotrophic lakes (Suttle et al. 1990; Stets and Cotner 2008). Heterotrophic bacteria showed higher affinities for limiting inorganic nutrients compared to phytoplankton (Jansson et al. 2007). Although organic matter with high molecular weight is normally less biolabile for bacteria than that with low molecular weight, adding nutrients may improve the bacterial utilisation of high-molecular-weight DOM and thereby the growth and nutrient requirement of bacteria (Zoppini et al. 1998; Thompson and Cotner 2020). Strong nitrate assimilation by heterotrophic bacteria occurs in freshwater receiving large amounts of terrestrial inputs and high nitrate concentration (Caraco et al. 1998). The TDOM bioavailability in eutrophic lakes is particularly important due to its effect on nutrient assimilation by heterotrophic bacteria. However, the present understanding about the combined effects of nutrients and photodegraded TDOM on bacterial abundance and particularly community structure is still rudimentary. We hypothesize that bacterial abundance and community structure will vary in response to different levels of photodegraded terrestrial humic acids and nutrient concentrations. To better assess the bacterial abundance and community structure response to photodegraded humic acids in eutrophic lakes, we exposed humic acids extracted from soil adjacent to the studied lake to UV lamp irradiation under different irradiation intensities. The optical characteristics of the products were analyzed. The photodegraded humic acids were used for bacterial incubation to determine the heterotrophic bacterial abundance and community composition. Specifically, we test the hypotheses that humic acids underwent strong irradiation intensity and increased nutrient concentrations can increase bacterial abundance and alter bacterial community composition. Our study has important implications for understanding the role of bacteria in the biochemical cycle of terrestrial organic matter in lakes.

Methods

Studied lake and sampling

Lake Xuanwu (32° 04′ N, 118° 47′ E) is located in Jiangsu Province with an area of 3.7 km^2 and an average water depth of 1.3 m. The general chemical characteristics of Lake Xuanwu are shown in Table S1. Lake water temperature was measured with a multi-parameter water quality measuring instrument (YSI 556 MPS). Total nitrogen (TN) and total phosphorus (TP) were determined by a Shimadzu UV2401 UV-Vis spectrophotometer using alkaline potassium persulphate digestion and the molybdenum blue method, respectively. The concentrations of ammonium (NH$_4$-N), nitrate (NO$_3$-N) and soluble phosphorus (PO$_4$-P) were measured using standard methods (Greenberg et al. 1992). By using water samples filtered through Whatman glass fibre GF/C filters, chlorophyll a (Chl a) concentration was determined by extraction with ethanol (90%) and spectrophotometric analysis at 665 nm and 750 nm (Chen and Gao 2000). The concentrations of dissolved organic carbon (DOC) were analyzed with a total organic carbon analyzer (Torch, TELEDYNE TEKMAR).

The surrounding surface soil within 5 m near the lake was obtained with a shovel in May 2016 to extract humic acids for photochemical experiment. Lake water was sampled in July 2016. The mixed lake water at 0–2 m was collected with a 5-L acid-washed Niskin water sampler. Samples were placed in 5-L acid-washed polypropylene plastic bottles wrapped with aluminium foil to avoid irradiation and immediately brought back to the laboratory.

Photodegradation experiment

Soil humic matter was obtained according to the method from the International Humic Substance Society (Thurman and Malcolm 1981; Watanabe and Kuwatsuka 1991). Briefly, 10 g of sample was adjusted to pH 2 with 1 mol/L HCl. After shaking, the supernatant was separated from the residue, which was then neutralized with 1 mol/L NaOH to pH 7.0. In a nitrogen-purged environment, NaOH solution (0.1 mol/L) was added to obtain an extractant-to-soil ratio of 10:1. The alkaline suspension was settled overnight, and the supernatant was collected through centrifugation. The supernatant was acidified with 6 mol/L HCl to a pH of 1.0, and then the suspension was settled overnight. The suspension was centrifuged at 5000 rpm for 10 min and the precipitate, which was humic acids, was separated from fulvic acid supernatant subsequently re-dissolved and dialyzed through a column of XAD-8 resin.

Humic acid was dissolved in sterile-filtered Milli-Q water with 10 mg/L initial concentration because the concentration of humic acid in natural water can be up to 10 mg/L (Wang and Ma 2012). NaOH solution (0.5 mol/L) was added to adjust the pH to 8.0, close to the pH value in Lake Xuanwu. Photochemical irradiation was performed in nine glass reactors with 12 L volume containing 10 L humic acid solution at 25°C, which was wrapped with light-proof aluminium foil. The humic acid solution was irradiated with 20 and 40 W UV lamps (named 20 W and 40 W groups) with the emission spectrum of 365 nm and corresponding irradiation intensity of 75 $\mu W/cm^2$ and 117 $\mu W/cm^2$. These irradiation intensities fell into the range of solar irradiance recorded by a TriOS RAMSES radiometer at the water surface of Lake Xuanwu. The top open of glass reactors was sealed with Whirl-Pak bag which did not keep in touch with water and could transmit solar radiation at wavelengths above 220 nm (Holm-Hansen and Helbling 1993). The lamp hung over with a distance of 10 cm to humic acid solution. The control group was treated without UV lamp, and the bottle was also sealed with aluminium foil. Each group was prepared in triplicate. The exposed water was sampled every 2 days during 54 days experimental period.

The absorption spectra of the exposed water were measured under 220–900 nm at 1 nm intervals using a Lambda35 UV–Vis spectrophotometer (Perkin Elmer) with a 5-cm quartz cuvette. Absorbance at 460 nm (A_{460}) was normalized as m^{-1}. The ratio of attenuation coefficients at 420 and 665 nm (E_4/E_6) was also calculated according to the equation described by Bricaud et al. (1981).

Bacterial population measurement

To investigate the effect of humic acid photodegradation level on bacterial abundance, unexposed and exposed humic acid solutions under irradiation intensities of 20 and 40 W at day 54 were used as carbon source for bacteria. The treatments were also named as 20 and 40 W groups, respectively.

To explore the influence of nutrient concentration on bacterial abundance, bacterial culture experiment with nutrient addition was carried out in the dark and lasted 13 days. This experiment consisted of a control group and two treatment groups named group A and B. The control group was treated without extra nutrient addition. Nutrient addition groups A and B were supplied with 1.21 and 2.42 mg/L $NaNO_3$ and 0.04 and 0.08 mg/L KH_2PO_4, respectively. The exposed humic acid solution under 40 W UV lamp was selected as the carbon source for bacteria culture.

The collected lake water was filtered using a 1.2-μm polycarbonate membrane filter (Ø47 mm) and the filtrate was used as bacterial inoculum. The mixture of bacterial inoculum and humic acid solution at 2:8 v/v was placed in sterilized light-proof glass bottles with 12 L volume containing 10 L culture medium at 25°C for 13 days. The samples were designed in triplicates. All samples were collected daily into 5 mL sterilized bottles. Formalin was instantly added at 2% final concentration to preserve the samples, which were filtered through a 0.22-μm black polycarbonate membrane and then stained with 4′6-diamidino-2-phenylindole for 20 min in the dark (Porter and Feig 1980). Counts were made with a Zeiss fluorescence microscope (AxioCam-MRc-5). We counted over 20 fields per slide in duplicate.

Bacterial community composition

The bacterial sample was collected from 100 mL of water in each bottle on 0.2-μm polycarbonate filters (Millipore). Bacterial DNA was extracted and purified according to Wu et al. (2006). The bacterial 16S rRNA gene was amplified by PCR and sequenced using the Illumina MiSeq platform (Yuan et al. 2014). The V4-V5 hypervariable regions of bacterial 16S rRNA genes were amplified using the primers 27F (5′-AGAG TTTGATCMTGGCTCAG-3′) and 1500R (5′-AGAA AGGAGGTGATCCAGCC-3′) (Tamura et al. 2007). DNA was amplified with three replicates in a Gene Amp PCR-System 9700 (Applied Biosystems, Foster City, CA, USA). Cycling parameters were set as follows: an initial denaturation step at 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 55°C for 45 s and 72°C for 1 min and a final extension at 72°C for 1 min. The triplicate PCR products from each sample were pooled and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and then quantified with PicoGreen (BMG Labtech, Jena, Germany).

Quality control and processing of MiSeq raw reads were conducted according to the following steps: (1) removing bases with a base mass of less than 20 at the end of the sequence and removing possible adapter sequences and short sequences

with a length less than 100 using TrimGalore; (2) splitting the paired sequences obtained by double-end sequencing and merging paired-end reads into longer reads using FLASH2; (3) finding and removing the primers in the sequence using Mothur and deleting the sequence containing N base/homopolymer over 6 bp; (4) removing sequences with a total base error rate greater than 2 and sequences less than 100 in length using Usearch.

The average value of the percentage of merged sequences in the total sequence number (3,919,453 clean reads) was 87.0%. Sequences with >97% similarity cut-off were clustered into one operational taxonomic unit (OTU) according to the UCLUST algorithm (Edgar 2010). A PyNAST with a relaxed neighbour-joining tree built using FastTree was employed to align a representative sequence for each phylotype (Price et al. 2010). The representative sequences of each OTU were classified into taxonomic groups referring to the typical freshwater bacterial database (Rohwer et al. 2017) and the RDP bacterial database (release 11.5). The alpha diversity was assessed using Shannon and Chaol diversity (Paul and Josephine 2004).

Data analysis

To analyze the photodegradation rate of humic acids irradiated with different irradiation intensities, we calculated the apparent rate constant (k_{app}) based on absorption coefficient. The rate equation used is $\ln C_0/C = k_{app}\ t$, where C_0 represents the initial concentration expressed as absorption coefficient at 460 nm (A_{460}) (Corin et al. 1996; Stedmon et al. 2000; Zhang et al. 2009), and C indicates A_{460} during photochemical reaction. The ratio of absorption coefficient at 460 and 660 nm (E_4/E_6) was determined to investigate the aromatisation level. A low E_4/E_6 value represents a high aromatisation level (Polak et al. 2009). The apparent rate constant (k_{app}) based on E_4/E_6 was also analyzed.

Significant differences among bacterial abundance and richness were determined by one-way analysis of variance (one-way ANOVA) with a p-value of 0.05 with the Statistical Program for Social Sciences (SPSS) 16.0 software. The effects of culture duration and irradiation intensity or nutrient dose on the bacterial diversity indices were analyzed by two-way ANOVA.

Results

Photodegradation of humic acids

During the 54-day exposure of soil humic acids, the A_{460} of the 20 W group changed from 7.83 to 5.48 m^{-1}, whereas the value in 40 W group decreased from 7.83 to 4.74 m^{-1} (Fig. S1). The value of A_{460} in the control group was rather stable (7.83–7.57 m^{-1}). The gradual decline in A_{460} in the 20 and 40 W groups represents a decrease in humic acid concentration. Moreover, the decreasing level of A_{460} in the 40 W group was more remarkable (39.52%) than that in the 20 W (30%) and control (3.36%) groups. In the present work, the ratio of E_4/E_6 ranged from 6.83 (initial) to 5.34 (54-day exposure) for the control group, 6.83 to 3.68 for the 20 W group and 6.83 to 2.76 for the 40 W group (Fig. S1). The kinetic constant (k_{app}) calculated with A_{460} in the 40 W group (0.0065 d^{-1}) was higher than that in the 20 W (0.0041 d^{-1}) and control (0.0002 d^{-1}) groups (Fig. 1a). This finding indicated a higher degradation rate of humic acid in the 40 W group than that in the 20 W group. The slope of the $\ln(E_4/E_6)$ was substantially different between treatments (Fig. 1b).
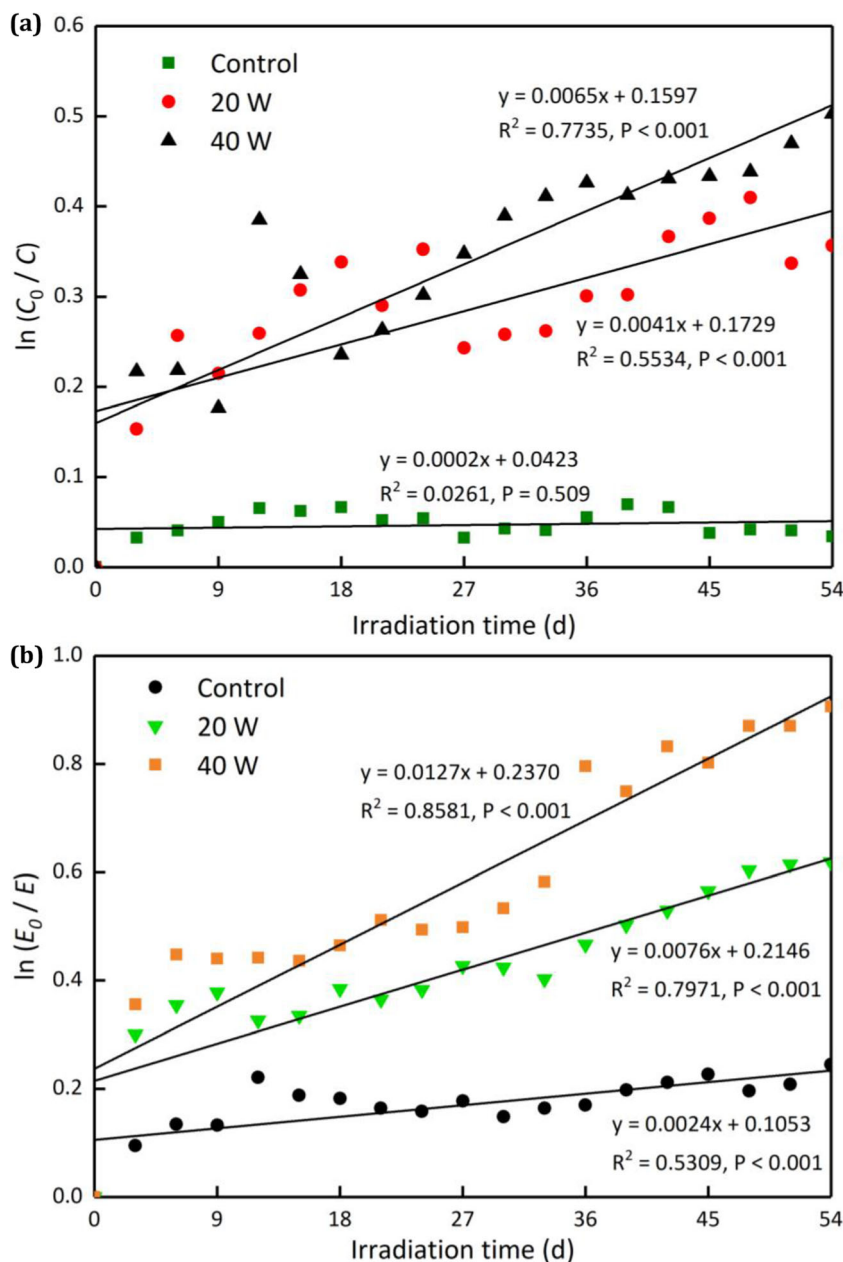
Bacterial abundance

The abundance of bacteria incubated with irradiated/non-irradiated humic acids shows a bimodal pattern (Fig. 2a). The first peak abundance of bacteria was $1.82\ (\pm0.10) \times 10^7$ (day 1), $3.74\ (\pm0.25) \times 10^7$ (day 2) and $4.77\ (\pm0.23) \times 10^7$ cells/mL (day 2) for control, 20 W and 40 W groups, respectively. The second peak appeared at day 6 of incubation time, with the abundance of $7.33\ (\pm2.37) \times 10^6$, $1.82\ (\pm0.46) \times 10^7$ and $1.88\ (\pm0.37) \times 10^7$ cells/mL for control, 20 W and 40 W groups, respectively. The abundance of bacteria incubated with humic acids exposed under high UV irradiation intensity was significantly higher than that incubated with humic acids under low UV irradiation intensity and with non-irradiated humic acids ($p < 0.001$).

Except for irradiation intensity, N and P affected the utilisation of humic acid breakdown products in the absence of irradiation. The abundance of bacteria also shows a bimodal pattern (Fig. 2b). The first maximum abundance of bacteria was $2.00\ (\pm0.15) \times 10^7$, $3.39\ (\pm0.16) \times 10^7$ and $4.03\ (\pm0.34) \times 10^7$ cells/mL at day 2 for control group, group A and group B, respectively. The second maximum abundance of bacteria reached $2.21\ (\pm0.08) \times 10^7$, $3.00\ (\pm0.26) \times 10^7$ and $3.51\ (\pm0.16) \times 10^7$ cells/mL at day 4 for control group, group A and group B, respectively. The concentrations of NH_4^+, NO_3^- and PO_4^{3-} were measured after day 2 to day 7 of bacterial incubation. The concentrations of NH_4^+ in three groups fluctuated with a slight increase at the first 2 days, followed by a small drop and then the change repeated once again (Fig. S2). The NO_3^- concentration of groups A and B slightly increased at the first 2 days and then continuously decreased from 1.48 to 0.25 mg/L and from 2.64 to 0.83 mg/L, respectively, whereas that of control group exhibited only a slight change. In addition, the concentration of PO_4^{3-} of groups A and B sharply decreased at the first 2 days, increased and then decreased from 14.71 to 2.37 µg/L and from 29.20 to 13.23 µg/L. Almost no change was observed for the PO_4^{3-} concentration of the control group.

Fig. 1 Exponential fitting for apparent rate constant (k_{app}) based on absorption coefficient (**a**) and E_4/E_6 (**b**). C_0 indicates the initial A_{460} and C indicates A_{460} during photochemical reaction. E_0 indicates the initial E_4/E_6 and E indicates E_4/E_6 during photochemical reaction
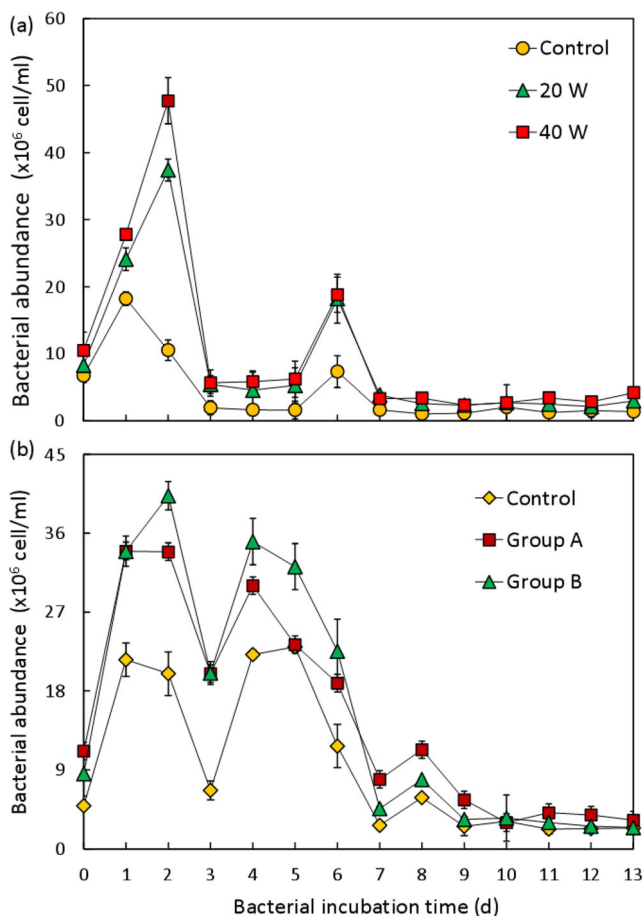

Bacterial community structure

The relative abundances of the dominant phyla and genera of bacteria incubated by humic acids irradiated under different irradiation intensities were shown in Figs. 3 and 4 and Fig. S3, respectively. Among the phyla, *Proteobacteria* was the dominant population, followed by *Bacteroidetes*, *Actinobacteria* and *Firmicutes*; moreover, the relative abundance of *Armatimonadetes* significantly increased in both treatments compared with the control on day 13 (Fig. 3).

For bacteria incubated with non-irradiated humic acids (control group), the relative abundance of *Aquabacterium*, *Flavobacterium* and *Limnobacter* varied from 18.92 ± 5.09 to $30.96 \pm 4.18\%$, 10.53 ± 2.33 to $0.94 \pm 0.15\%$ and 2.02 ± 0.65 to $24.27 \pm 8.44\%$, respectively, from day 2 to day 13 (Fig. S3). For bacteria incubated with humic acids from the 20 W group, the relative abundance of *Aquabacterium* and *Limnobacter* varied from 14.22 ± 9.46 to $14.8 \pm 10.57\%$ and 2.23 ± 0.66 to $20.84 \pm 6.45\%$ from day 2 to day 13, respectively. For bacteria incubated with humic acids of the 40 W group, the relative abundance of *Aquabacterium* and *Flavobacterium* was 7.33 ± 3.58 to $16.68 \pm 9.03\%$ and 16.22 ± 9.61 to $1.05 \pm 0.83\%$, respectively, from day 2 to day 13. In addition, *Armatimonadetes-gp4* and *Methyloversatilis* were only identified in the samples after 13 days of incubation. The abundance of *Aquabacterium* in

Fig. 2 **a** Abundance of bacteria incubated by humic acid irradiated under different irradiation intensities. **b** Abundance of bacteria incubated by humic acid irradiated under 40 W UV lamp combined with different nutrient concentrations

both the 20 W and 40 W groups was significantly lower than the control ($p < 0.001$). The genus of bacteria was amended with humic acids that were irradiated with different intensities (Fig. 4).

Under the condition of nutrient addition, the relative abundances of the dominant phyla and genera of bacteria incubated by humic acid irradiated at 40 W were shown in Figs. 5 and 6 and Fig. S4, respectively. For the phyla, the dominant population was *Proteobacteria*, followed by *Bacteroidetes*, *Actinobacteria* and *Planctomycetes* (Fig. S5 and S4). This dominant population is quite similar with the phyla of bacteria incubated by humic acid irradiated under different irradiation intensities. The genera mainly included *Novosphingobium*, *Armatimonadetes_gp4*, *Sediminibacterium* and *Methylobacterium* (Fig. S4).

For bacteria incubated with humic acids irradiated with 40 W lamp, the relative abundance of *Armatimonadetes-gp4* and *Sediminibacterium* was obviously higher than that in the control group. The relative abundance of these two genera greatly decreased after 2 days of incubation and then increased at day 13 (Fig. 6 and Fig. S4). *Armatimonadetes-gp4* and

Sediminibacterium correspondingly exhibited an initial proportion of 2.03% and 2.88% in the control group, 7.41% and 8.62% in group A and 5.6% and 8% in group B. After 2 days of incubation, their corresponding proportion increased to 5.62% and 3.41% in the control group, 10.43% and 7.32% in group A and 11.63% and 9.53% in group B. After 13 days of incubation, the relative abundance of *Sediminibacterium* in groups A and B was 5.94% and 6.18%, respectively. *Limnobacter* was not found at first in the control group, and its proportion in groups A and B was 1.49% and 1.84%, respectively. The proportion of *Limnobacter* after 2–13 days of incubation was higher than the initial value. Moreover, the highest proportion occurred in group B.

A Venn diagram of obtained OTUs shows 20 and 40 W groups and the control shared 242 and 215 core OTUs at days 2 and 13, respectively, accounting for 54.1% and 47.9% of the total OTUs, respectively (Fig. 7a, b). In the nutrient addition experiment, group A and B and the control shared 347 and 301 core OTUs at days 2 and 13, respectively, accounting for 52.3% and 45.5% of the total OTUs, respectively (Fig. 7c, d).

Shannon and Chaol diversity

The Chaol and Shannon-Wiener indices for bacteria incubated with non-irradiated humic acids (control group) at day 2 were the highest, followed by the 20 W group and then the 40 W group (Fig. 8a). Chaol and Shannon-Wiener indices in the control group were higher than those in groups A and B during the whole 13 days of incubation ($p < 0.005$) (Fig. 8b). These two indices presented a significant decrease from the initial stage to day 2, particularly in group A. Two-way ANOVA results indicated that culture duration always affected the bacterial diversity indices in both the photodegradation experiment and the nutrient addition experiment (Tables 1 and 2). However, the differences in the effects of irradiation intensity and nutrient dose on the bacterial diversity indices were not significant except for the effect of irradiation intensity on Chao1 indices.

Discussion

Bacteria are considered to have the highest number of species in lakes and are highly sensitive to environmental shift due to their rapid growth; thus, they play a key role in aquatic ecosystems. It is well acknowledged that substrates and trophic level exert important effects on bacterial growth and abundance. The bioavailability of substrates mainly depends on their molecular structure and weight, which can be greatly affected by photochemical process (Su et al. 2015). In this study, UV irradiation decreased the concentration of humic acids, as evidenced by the decrease in A_{460} along with irradiation intensity and time (Fig. 1). The decreasing ratio of E_4/E_6
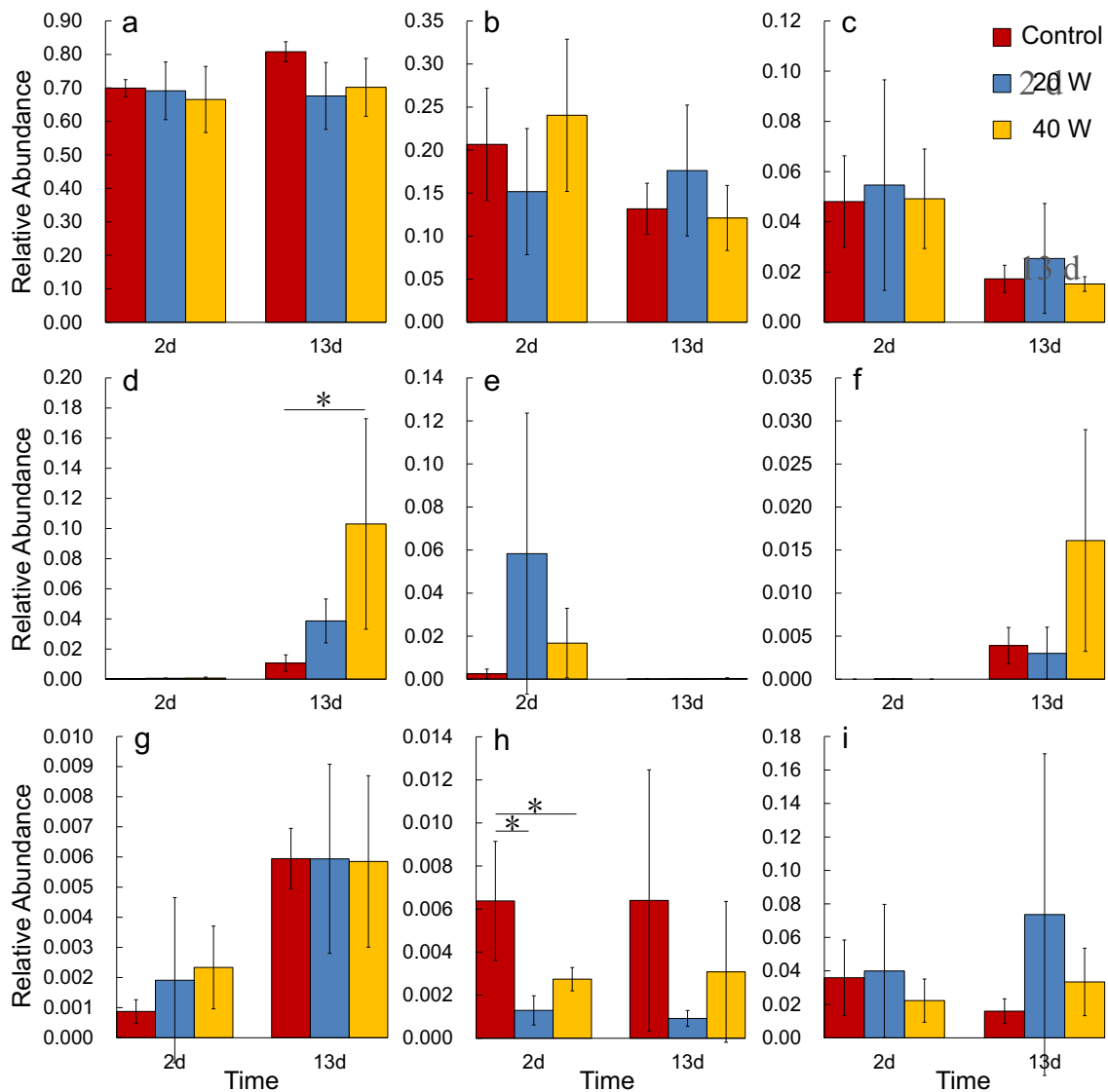
Fig. 3 Bacterial community structure at phyla level (the top 8 bacterial phylum) incubated by humic acid irradiated under different irradiation intensities 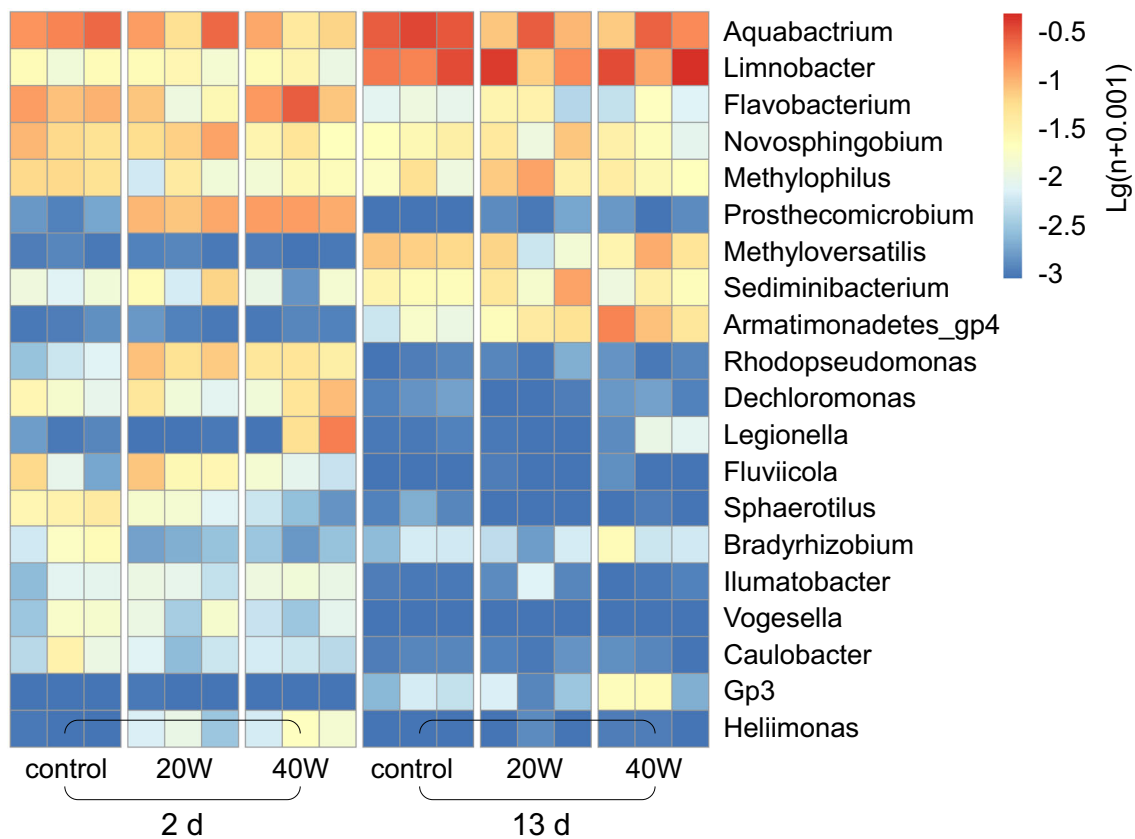after 2 and 13 days incubation. **a** Proteobacteria. **b** Bacteroidetes. **c** Actinobacteria. **d** Armatimonadetes. **e** Cyanobacteria. **f** Acidobacteria. **g** Verrucomicrobia. **h** Planctomycetes. **i** others

suggested that the aromatisation level of humic acids increased, which is ascribed to a strong UV photo-oxidation effect. Humic acids are susceptible to UV radiation; thus, a breakdown of chromophoric groups attacked by reactive species, such as hydroxyl radicals, can modify its molecular structure (Corin et al. 1996; O'Sullivan et al. 2005; Varanasi et al. 2018). However, macromolecular humic acids are not completely mineralized during photo-oxidation reactions; rather, it is cleaved into small molecules, including carboxylic acids and carbohydrates, which are readily assimilated by aquatic bacteria (Li et al. 1996; Anesio et al. 2005).

Self-shading effects during the experiment would be not avoided due to the big volume of sample. Nevertheless, the effects of self-shading were limited deduced from the experimental result that there was obvious difference between different light intensity treatments. Bimodal pattern in bacterial abundance was found in both treatments and the control. Similar pattern was also reported by Osinga et al. (1997) and Zhou et al. (2021). The bimodal pattern would be due to the interactions between bacteria but counting error was not being excluded.

Our findings revealed that the abundance of bacteria is in the order that 40 W group > 20 W group > the control (Fig. 2). Generally, bacteria tend to use organic carbon with relatively simple molecular structure. The growth efficiency of bacteria utilising DOC with low molecular weight (16–66%) is significantly higher than that with high molecular weight (8–39%) (Münster and Chróst 1990; Amon and Benner 1996). Thus, this result indicated that humic compounds after irradiation became

Fig. 4 Bacterial community structure (the top 20 bacterial genera) at genus level incubated by humic acid irradiated under different irradiation intensities after 2 and 13 days incubation

more bioavailable and higher light intensity was more likely to decompose humic acids.

DOC generally plays a key role in bacterial community composition (Bouvy et al. 2011). The photodegradation of humic acids not only enhanced its bioavailability for bacteria but also thrives on competition between bacteria, as evidenced by different bacterial community compositions in different irradiation groups (Fig. 3a and 3b). For instance, the dominant bacteria were *Aquabacterium* for the control group, *Aquabacterium* and *Limnobacter* for the 20 W group and *Flavobacterium* and *Limnobacter* for the 40 W group. Moreover, *Limnobacter* was predominant at day 13 and *Armatimonadetes-gp4* and *Methyloversatilis* began to appear at the same time. Therefore, the utilisation of particular organic matter can lead to significant differences in bacterial community structure (Cottrell and Kirchman 2000).

High bacterial abundance in nutrient addition groups within day 2 (Fig. 2) corresponds to a rapid decrease in PO_4^{3-} concentrations (Fig. S1), implying that bacterial abundance was enhanced by phosphate. Nutrient, particularly phosphorus, is a limiting factor for bacterial growth and utilisation of organic carbon (Zoppini et al. 1998; Danger et al. 2007). Some evidence support that a low C:N or C:P ratio caused by nutrient addition can potentially improve the bioavailability of organic carbon for bacteria (del Giorgio and Cole 1998).

During humic acid photodegradation, additional tyrosine-like and tryptophan-like fluorescence products are created with relatively low C:N ratio (Su et al. 2017). In the present study, NH_4^+ and NO_3^- concentration increased from the initial stage to day 2 and the increasing rate of NH_4^+ was in the order that group B > group A > control. At this point, the bacterial abundance was also in the order that group B > group A > control. This finding is probably related to the regeneration of N through bacteria utilising dissolved organic matters. The lower the C:N ratio for organic matter, the more organic matter is available. More bacteria can multiply to break down more organic matter and turn it into ammonia (Goldman et al. 1987). In addition, heterotrophic bacteria require additional energy to transform nitrate into ammonia-N, which is easier for bacteria to utilize ammonia-N than nitrate (Vallino et al. 1996; Chu et al. 2005). Nonetheless, nitrate could become important for bacterial metabolism and growth in a condition of lacking of ammonia-N (Karayanni et al. 2019; Kirchman et al. 1994; Jiang and Jiao 2016), as evidenced from the decreasing concentration of NO_3^- after day 2 in this work. These results implied that both nitrification and denitrification were enhanced with the increased concentration of inorganic N.

It was reported that increasing nutrient input has a significant influence on bacterial community composition (Tang
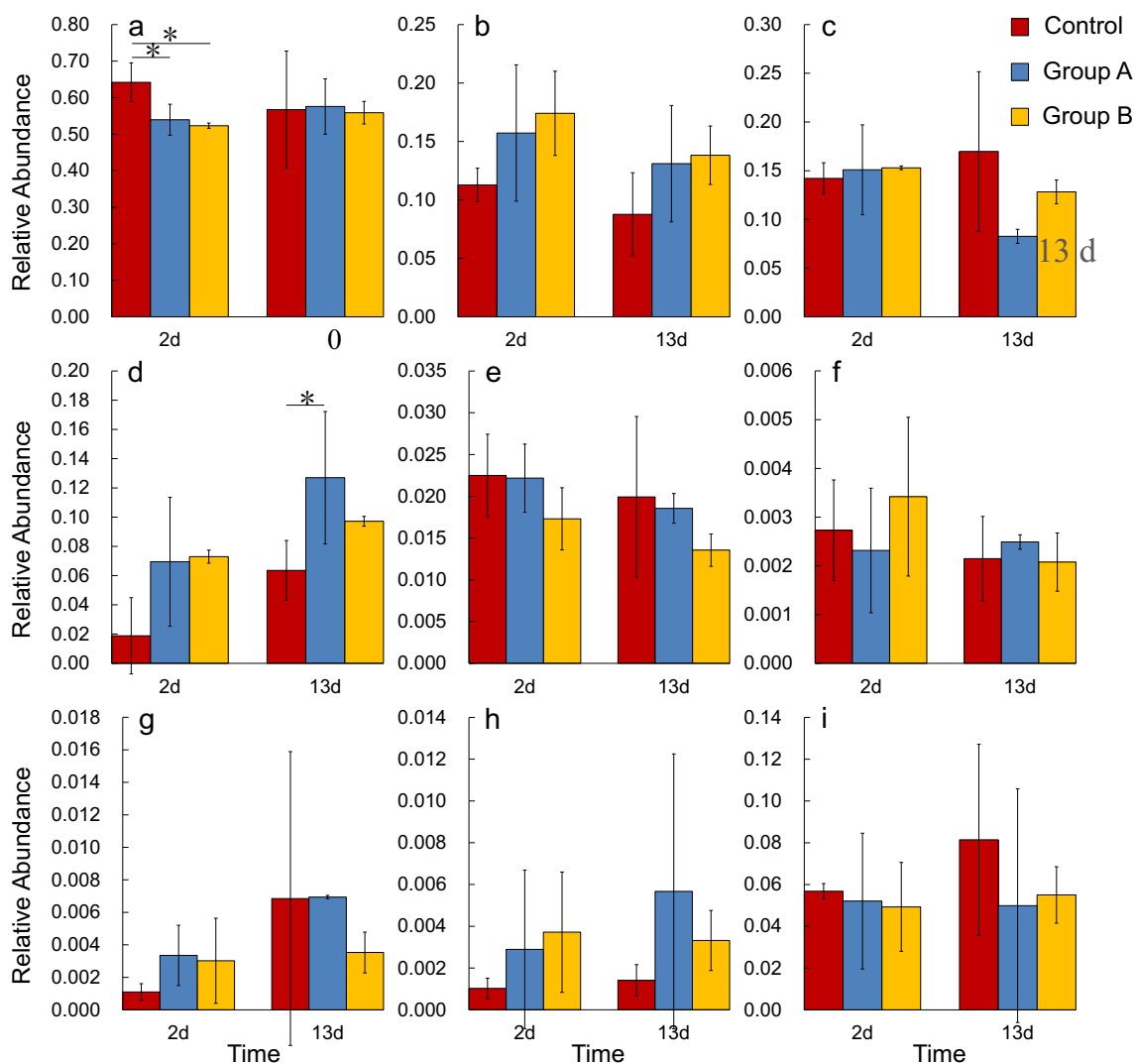
Fig. 5 Bacterial community structure at phyla level (the top 8 bacterial phylum) incubated by humic acid irradiated under 40 W UV lamp combined w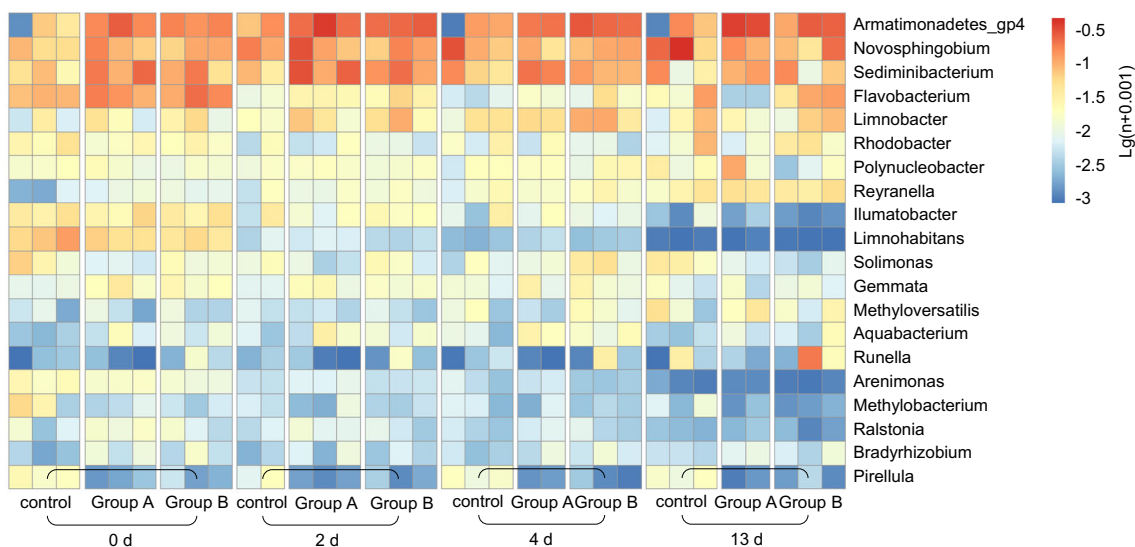ith different nutrient concentrations after 2 and 13 days incubation. **a** Proteobacteria. **b** Bacteroidetes. **c** Actinobacteria. **d** Armatimonadetes. **e** Planctomycetes. **f** Acidobacteria. **g** Cyanobacteria. **h** Spirochaetes. **i** others

et al. 2012). In this work, the dominant population was *Flavobacterium* and *Novosphingobium* for the control group and *Armatimonadetes-gp4* and *Sediminibacterium* for groups A and B (Fig. 3c and 3f), implying that the abundance of *Armatimonadetes-gp4* and *Sediminibacterium* increased with rising nutrient concentration. Besides, abundance of *Hydrocarboniphaga* significantly increased with the increasing nitrogen concentration. This would be due to that many species of *Hydrocarboniphaga* can utilize ammonia-N and nitrate as nitrogen source (Palleroni et al. 2004).

Zheng et al. (2008) reported that *Firmicutes* was the dominant population during *Microcystis* spp. bloom in Lake Xuanwu, followed by *Bacteroides* and *Proteobacteria*, while *Proteobacteria* grew fast to be the dominant population during the bloom declining period, followed by *Firmicutes*. Different bacterial community composition during phytoplankton bloom period reflected the adaption of the bacterial physiological characteristics to the environment. For instance, *Bacteroides* and *Firmicutes* are widely distributed in anaerobic environment and they have capabilities to decompose complex organic polymer substances, such as carbohydrates and proteins (Dworkin et al. 2006), and *Hydrocarboniphaga* as a novel member of the γ-*Proteobacteria* is active in alkane and aromatic hydrocarbon degradation (Palleroni et al. 2004). In this work, the relative of abundance of *Bacteroides* (*Flavobacterium*) at day 2 was much higher than at day 13 for both irradiated and control groups (Fig. S2c). This is because complex organic substances had a relative high concentration at day 2, after 13-day incubation, and complex organic substances were decomposed to labile substances.

The initial bacterial community contained a very low amount of typical freshwater microbes (Fig. 3, Fig. S2 and Fig. S3), but was rather composed of copiotrophs which are fast-growing microbes that overgrow others in manipulated

Fig. 6 Bacterial community structure at genus level (the top 20 bacterial genera) incubated by humic acid irradiated under 40 W UV lamp combined with different nutrient concentrations after 2 and 13 days incubation

Fig. 7 Venn diagram of obtained OTUs in various treatments. **a** Photodegradation experiment at day 2. **b** Photodegradation experiment at day 13. **c** Nutrient addition experiment at day 2. **d** Nutrient addition experiment at day 13
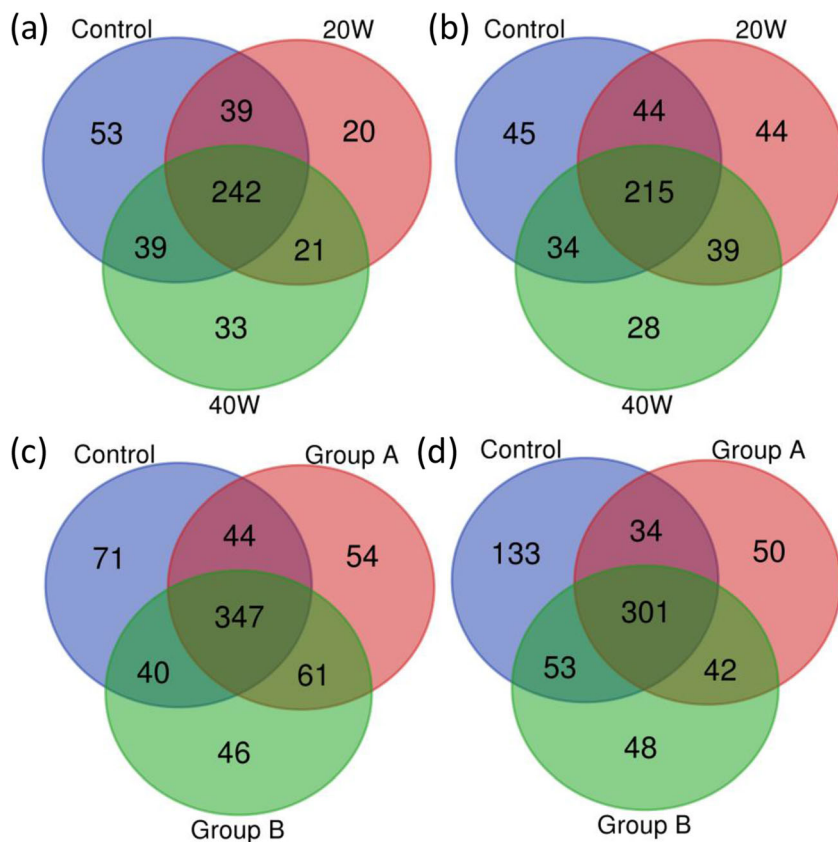

Table 1 Summary of two-way ANOVA of the effects of culture duration and irradiation intensity on the bacterial diversity indices in the photodegradation experiment

Parameter	Source of variations	SS	df	MS	F	Sig.
Chao1	Culture duration	2969.07	1	2969.07	4.883	0.047
	Irradiation intensity	7676.02	2	3838.01	6.312	0.013
	Culture duration × irradiation intensity	1193.55	2	596.77	0.981	0.403
Shannon	Culture duration	0.94	1	0.94	18.814	0.001
	Irradiation intensity	0.12	2	0.06	1.203	0.334
	Culture duration × irradiation intensity	0.16	2	0.08	1.552	0.252

Table 2 Summary of two-way ANOVA of the effects of culture duration and nutrient dose on the bacterial diversity indices in the nutrient addition experiment

Parameter	Source of variations	SS	df	MS	F	Sig.
Chao1	Culture duration	72,081.67	3	24,027.23	13.632	<0.001
	Nutrient dose	8316.90	2	4158.45	2.359	0.119
	Culture duration × nutrient dose	5819.96	6	969.99	0.550	0.764
Shannon	Culture duration	0.68	3	0.23	4.382	0.015
	Nutrient dose	0.07	2	0.03	0.649	0.533
	Culture duration × nutrient dose	0.05	6	0.01	0.175	0.981

Fig. 8 Chaol and Shannon-Wiener indices for bacteria in photodegradation experiment (**a**) and nutrient addition experiment (**b**). Different letters above bars indicate significant differences in each indices between treatments according to one-way ANOVA ($p < 0.05$)
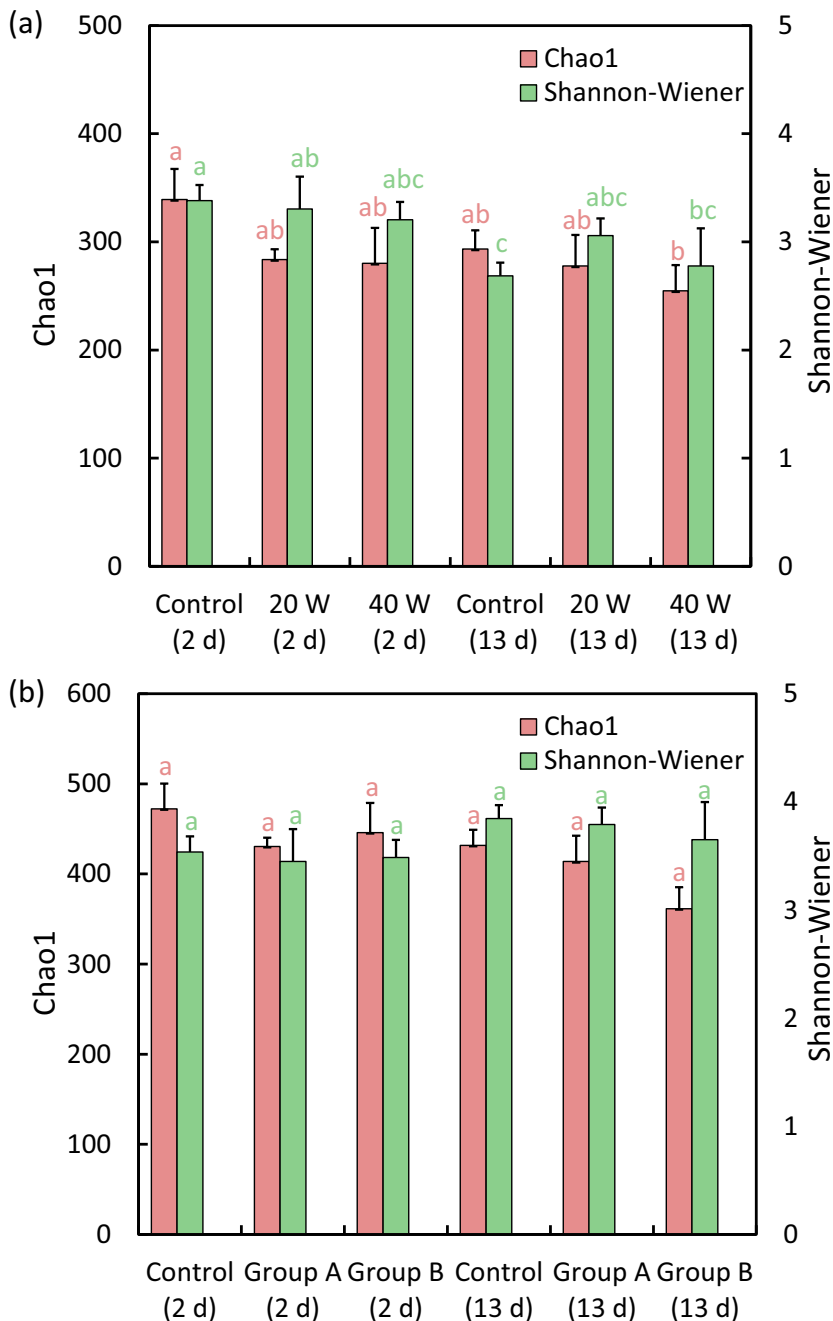
samples. This could be partly because the classical bottle effect affects the microbial community if water samples are kept in the lab for a long time (13 days in this work). Furthermore, a filtration through 1.2-μm filters might also include small protists (nanoflagellates) that feed on bacteria and thus change the composition of the community during the experiments. However, the bottle effect and small protists should exist in each group and their effects on the microbial community could be reflected in the control group. We observed that the change of microbial community in 20 and 40 W groups and groups A and B was very much different from their corresponding control group. Consequently, we believe that this difference of microbial community between control and the other groups was mainly caused by the photodegraded humic acids or nutrients.

Conclusions

Our results provide evidence that the exposure of terrestrial humic acids to UV light and the concentration of nutrients significantly affect the heterotrophic bacterial abundance and community composition in Lake Xuanwu. Bacteria showed relatively high abundance through utilising humic acids irradiated under high UV intensity, revealing that bacteria tend to utilize organic carbon with relatively simple molecular structure. A high concentration of nutrients exhibited an important effect on bacterial abundance as well. The increasing nutrient concentration resulted in an overall reduction of the richness and diversity of bacteria. Our findings offer important implications to better understand how freshwater bacteria will change in response to increasing input of terrestrial matters.

Declarations

Ethics approval and consent to participate Not applicable.

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

Conflict of interest The authors declare no competing interests.

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