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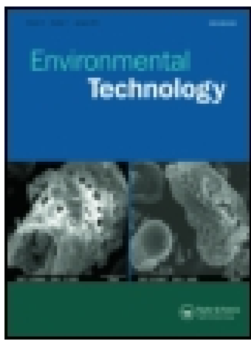

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Functional sustainability of nutrient accumulation by periphytic biofilm under temperature fluctuations

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Abstract

Temperature can fluctuate widely between different seasons, and this may greatly impact many biological process. However, little is known about its influence on the functioning of benthic microbial communities. Here we investigated the nutrient accumulation capability of periphytic biofilm under temperature fluctuations (17-35 °C). Periphytic biofilm maintained the same nutrient accumulation capacity after experiencing the "warming-hot-cooling" temperature fluctuation under both lab and outdoor conditions as those without temperature disturbance. In response to temperature increase, both community composition and species richness changed greatly and the increase in biodiversity was identified as being the underlying mechanism boosting the sustainable function in nutrient accumulation, indicating zero net effects of community changes. These findings provide insights into the underlying mechanisms of how benthic microbial communities adapt to temperature fluctuations to maintain nutrient accumulation capacity and elucidate that periphytic biofilm plays important roles in influencing nutrient cycling in aquatic ecosystems under temperature changes such as seasonal fluctuations.

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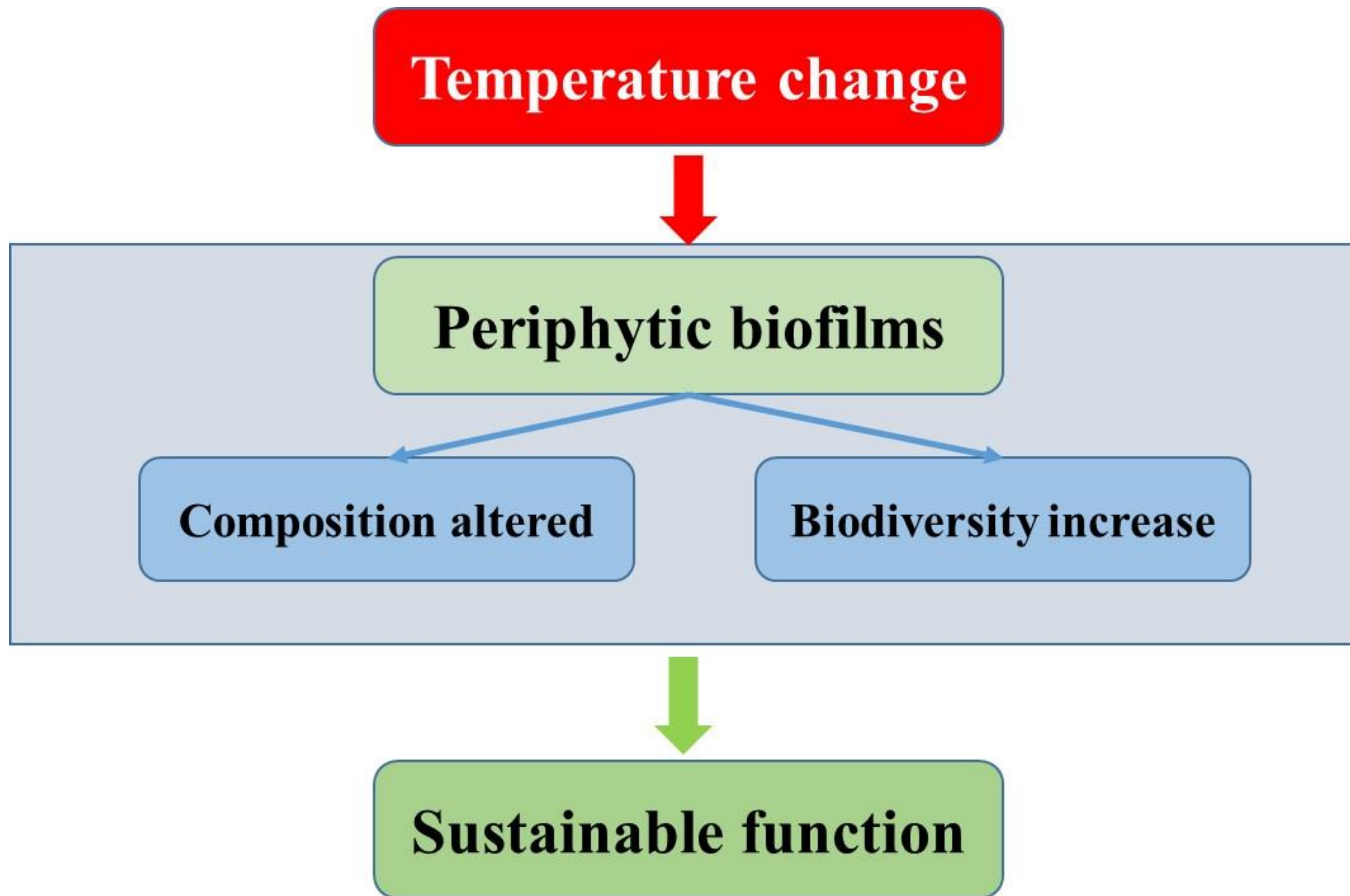

Keywords: Periphytic biofilm, Functional redundancy, Nutrient accumulation, Community structure

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1. Introduction

Climate events and seasonal changes have remarkable consequences on ecosystem processes [1, 2]. So far, considerable efforts have been devoted to understanding how temperature fluctuations, including daily and seasonal changes, influence the composition, structure, and diversity of microbial communities [2-5]. Compared to meso- and macro-communities, microbial communities with shorter generation times are more sensitive to disturbances but rarely recover to their original status when suffering from external stresses [6]. Consequently, these responses beg the question as to whether the microbial compositional shifts caused by temperature fluctuations will affect the associated ecosystem processes [7]. This is important for the functions of microbial communities. However, most current studies focus on the influence of temperature fluctuations on the microbial community composition, but rarely study the functions of a microbial community as a whole in response to temperature fluctuations [8, 9].

Periphytic biofilm, composed of autotrophic and heterotrophic microorganisms, is a typical benthic microbial community in shallow aquatic ecosystems, forming a layer between sediment and water [10]. The conditions at the sediment-water interface are characterized by steep chemical gradients, shaped by the interplay between physical, chemical, and microbial processes [11, 12]. Thus, as the essential layer for mass transport between sediment and water, periphytic biofilm impacts the biogeochemical cycles of nutrients at sediment-water interface [13-15]. As a community, periphytic biofilm functions in nutrient transformation through assimilation, precipitation, nitrification, denitrification and other pathways. As a species rich community, periphytic biofilm exhibits complex species interactions, and these can influence its collective functions by changing the community composition [16, 17]. In addition, there are function

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overlaps between different species in any species-rich microbial community and in a sense, this enhances the sustainable functions of a microbial community by acting as an 'insurance' [18].

Temperature change occurs naturally, either from morning to night, or from spring to winter and drives the variations in community populations [5, 19]. Associated with variations in community caused by temperature changes, the functions of periphytic biofilm may be enhanced, weakened or stay the same, but the underlying mechanisms still remains largely unknown [20, 21]. The rates of ecosystem processes depend highly on the species composition and metabolic activities [22]. For example, following temperature increase, the periphytic community may get dominated by thermophilic species associated with community structure changes. The metabolic activities such as nutrient uptake capacity of many individual microbial species slow down at a temperature higher than the optimal, resulting in deterioration in their nutrient accumulation capacity [16, 23]. Similarly, the temperature fall may weaken many metabolic activities and prompt the dominance of species preferring low temperature [23]. However, it is not clear whether the function of periphytic biofilm in nutrient accumulation will be affected by temperature fluctuations, or if the function can recover when the temperature returns to the original value.

Periphytic biofilm can adapt to a wide range of temperature (e.g. 4-50 °C), however, its sensitivity to temperature changes varies greatly [24, 25]. Thus, the temperature perturbation may have impacts through a network of direct and indirect pathways between individual species within the periphytic community. The outcomes of the temperature perturbation may be highly sensitive to the intensity of the interactions amongst microbial species [26]. García, Bestion, Warfield, et al [27] found that as temperatures departed from ambient conditions the exponent of the diversity-function relationship increased, meaning that more species were required to maintain ecosystem

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functioning under thermal stress. This leads to the question of how the community structure of periphytic biofilm influences nutrient accumulation functions.

Accordingly, it is hypothesized that the changes in the species-rich periphytic community can maintain sustainable ecosystem functions in nutrient accumulation as a whole community after seasonal temperature fluctuations. In this study, controlled indoor and outdoor experiments were conducted to elucidate that (i) periphytic biofilm can maintain nutrient accumulation capacity via changing its community composition, structure and diversity; and (ii) periphytic biofilm maintains its stability and sustainability in function species interactions.

2. Materials and methods

2.1 Preparation of periphytic biofilm

The periphytic biofilm and its attached sediment (about 5.0 cm in depth) were collected from Xuanwu Lake, Nanjing, East China, using a plastic pipe (diameter 12.0 cm). The properties of the sediment were as follows: pH 6.5-7.8, total nitrogen (TN) 36.28±2.75 mg/kg, total phosphorus (TP) 13.19±0.98 mg/kg, Labile-P 1.23±0.10 mg/kg, Fe/Al-P 4.16±0.29 mg/kg, Ca-P 7.24±0.43 mg/kg. The collected periphytic biofilm and attached sediment were placed into a glass tank (length × width × height = 30 cm × 20 cm × 50 cm) for further culture before use.

Hyper-eutrophic water (pH 7.9, NO_3^--N+NO_2^--N 1.73 mg/L, NH_4^+-N 1.53 mg/L, total inorganic phosphorus 0.2 mg/L, dissolved inorganic phosphorus 0.05 mg/L, chemical oxygen demand (COD) 190 mg/L) collected from the same lake was put into the tank and the water depth was kept at 35.0 cm by adding distilled water to compensate for losses due to evaporation.

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To maintain the dissolved oxygen (DO) content at 7.0-9.0 mg/L, air was continually supplied using an aerator (power: 12 W). The tanks were placed on the rooftop of the experimental building. Tanks were moved to a greenhouse when the outdoor temperature was less than 20 °C. During the experiment, the air temperature varied from 20 to 36 °C. To avoid contamination by insects, the tanks were covered with sheer nylon net (mesh < 0.2 mm). After two weeks, a thick brown layer (~ 0.5 cm) of periphytic biofilm had formed. Then, the periphytic biofilms were peeled off using sterile blades and washed using distilled water until no sediment was visible to the naked eye. Samples were filtered (20 mesh nylon webbing) until drip-free. Thereafter, the collected periphytic biofilms were used for the following experiments.

2.2 Laboratory experiment

To evaluate the effects of temperature fluctuation on community composition and nutrient accumulation capacity of periphytic biofilm, a controlled indoor experiment was conducted. Specifically, at the beginning of the experiment 10 g of wet periphytic biofilm (water content 90-95%, the same below) was placed in 250 mL BG-11 medium on top of 2 cm sterilized sand in 500 mL beakers. The beakers were then placed into an incubator. The temperature treatment and recovery were imposed by first increasing and then decreasing the air temperatures of the incubator, which was called temperature fluctuation (17-35-17°C) (Fig. 1a). Starting at 17 °C, the temperature was raised by 2.0 °C every 15 days to a maximum of 35 °C. Thereafter, the air temperature was decreased by 2.0 °C every 15 days back to 17 °C. The warming phase was defined as the phase with temperature increasing from 17 °C to 31 °C (day 0-105). The hot phase was the phase with temperature changing from 31 °C to 35 °C and back to 31 °C (day 105-165). The period with temperature decreasing from 31 °C to 17 °C was defined as the cooling phase (day 165-270).

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Temperatures for each regime were chosen based on the historical climatological data in Nanjing, China, where the main investigation of this study was performed. To ensure periphytic biofilm has enough time to acclimatise or adapt to increasing or decreasing temperature, an interval of 15 days between temperature changes was used. The light conditions were as follows: illumination 2800±10 Lux, with light/dark cycle of 12 h/12 h. To supply sufficient nutrient for periphytic biofilm growth and avoid degradation, the BG-11 medium was refreshed every 15 days. Meanwhile, the control was conducted by culturing periphytic biofilm at constant temperature (25±1 °C) under the same light and nutrient conditions as the treatment. Both the treatments and controls were performed in triplicate and the results presented as averages.

After experiencing the abovementioned temperature fluctuation, the 270-day old periphytic biofilms from both treatment and control were selected to evaluate their nutrient (i.e., nitrogen and phosphorus) accumulation capacity by using simulated hyper-eutrophic water. Briefly, 5.0 g of wet periphytic biofilm (water content 90-95%) was added to 150 mL of simulated hyper-eutrophic water, composed of macronutrients (20 mg/L $NaCO_3$, 150 mg/L $NaNO_3$, 4 mg/L K_2HPO_4, 75 mg/L $MgSO_4 \cdot 7H_2O$, 36 mg/L $CaCl_2 \cdot 2H_2O$), micronutrients (2.86 mg/L H_3BO_4, 1.81 mg/L $MnCl_2 \cdot 4H_2O$, 0.22 mg/L $ZnSO_4$, 0.39 mg/L Na_2MoO_4, 0.079 mg/L $CuSO_4 \cdot 5H_2O$, 4.94 mg/L $Co(NO_3)_2 \cdot 6H_2O$) and organic matter (6 mg/L citric acid, and ammonium ferric citrate). The pH was adjusted to 7.0 by using 0.1 M NaOH or HCl solution as required. DO during experiment was maintained at 7.5-8.5 mg/L by an aerator. The experimental conditions were as follows: illumination 2800±10 Lux, with light/dark cycle of 12 h/12 h, air temperature 25±1 ºC. The hyper-eutrophic water was sampled daily to measure TN and TP concentrations for 7 days.

2.3 Inter-region transplant experiment

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To investigate and compare the nutrient accumulation capacity of periphytic biofilm cultured under different climate conditions, an inter-region transplant experiment was conducted in three cities in the temperate zone in China: (1) Nanjing, East China (annual average air temperature 15.3 ºC, month average 2.3-29.2 ºC); (2) Kunming, Southwest China (annual average temperature 16.4 ºC, month average 4.2-24.5 ºC); and (3) Xi'an, Northwest China (annual average temperature 13.3 ºC, month average -1.2-30.3 ºC).

For the inter-region transplant experiment, the periphytic biofilm cultured in Nanjing was used. Firstly, the periphytic biofilm was relocated to Kunming and cultured for one year (from June 11, 2013 to June 11, 2014). Thereafter, the biofilm was transplanted to Xi'an and cultured for another year (from June 12, 2014 to June 12, 2015) (Fig. 2a). The compositions of the culture media for the biofilms in these three cities were the same. Specifically, 5 L of BG-11 medium on top of 2 cm sterilized sand in glass tanks, were inoculated with 40 g of wet biofilm and placed outside on the rooftop under natural conditions. To maintain sustainable nutrient supply, 5 mL stock solutions (1000 times concentration of the culture medium) of BG-11 medium were added into the tank every 30 days and distilled water was added to 5 L level every 7 days to compensate for water evaporation. During rainy days, the tanks were covered with transparent plastic sheeting to avoid rain disturbance. When the biofilms were to be relocated, 40 g of biofilm was sampled, and transported to the next site in an ice box.

At the end of each transplant experiment, the nutrient accumulation capacity of the periphytic biofilms was evaluated. Briefly, 5.0 g of wet periphytic biofilm was added to 150 mL of simulated hyper-eutrophic water with the same chemical composition as the lab experiment. During the 7 days culture of the biofilm, TN and TP concentrations of the hyper-eutrophic water were measured

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every 24 hrs. The culture conditions (light regime, DO and air temperature) were the same as for the lab experiment.

2.4 Sampling and analyses

To determine carbon metabolic and enzymatic activities for the lab experiment, biofilm samples were collected every 15 days. The periphyton samples collected on day 1 (warming phase), day 135 (hot phase), and day 270 (cooling phase) were used for Biolog^{TM}, phospholipid fatty acid (PLFA) and 16S rRNA sequencing analyses. This was done in triplicate and the results were presented as averages ±S.D.

Biofilm carbon metabolic profiles. Biofilm samples from different phases were collected to determine their microbial carbon metabolic activities using Biolog EcoPlates (Biolog Inc., Hayward, CA, USA) [28]. The Biolog EcoPlates consisted of 96-well microplates, containing 31 different carbon sources plus a blank well including three replications. Carbon sources were subdivided into six groups of substrates including carbohydrates, carboxylic acids, amino acids, polymers, phenolic acid, and amines/amides [29]. For each treatment, 2 g of wet biofilm (moisture 85-90%) was suspended in 50 mL distilled water and shaken for 30 min at 200 rpm in an incubator at 25 °C. Thereafter, 1 mL of microbial solution was diluted to 20 mL, and 150 µL of the diluted solution was added to each well of the EcoPlates, which were incubated at 25 °C, with the average well color development (AWCD) being determined using a Biolog Microplate Reader at 590 nm every 24 h for 144 h. The degree to which a particular substrate is utilized is quantified by measuring the intensity of color change caused by incorporation of tetrazolium dye into a respiring bacterial community. Optical density (OD_{590}) value from each well was corrected by subtracting the blank well values.

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PLFA profiles. We characterized microbial biomass by PLFA analysis following a modified method [30]. Briefly, total lipids were extracted from 2 g of freeze-dried biofilm samples with a chloroform-methanol-citrate buffer mixture (1:2:0.8, *v*/*v*/*v*) and separated into neutral, glyco- and phospholipids by a silica acid column. Phospholipids were subjected to a mild alkaline methanolysis, and the fatty acid methyl esters were quantified by a HP 6890 Series gas chromatograph instrument (Hewlett Packard, Wilmington, DE, USA). Identification was performed using bacterial fatty acid standards and MIDI peak identification software (Microbial ID Inc., Newark, DE, USA). Microbial biomass was calculated by summing the abundance of specific biomarkers and expressed as nmol PLFA g^{-1} dry biofilm. The following PLFAs were representative markers of the specific groups: Gram-negative bacteria (cyclopropyl bacteria and unsaturated PLFAs) , Gram-positive bacteria (iso- and anteiso-branched PLFAs) , actinomycetes (10Me PLFAs) , fungi (18:1ω9c and 18:2ω6, 9c) and protozoan (20:2ω6, 20:3ω6 and 20:4ω6) [31]. The sum of Gram-positive bacteria, Gram-negative bacteria, and non-specific bacteria was expressed as the bacterial biomass.

Community composition. To determine the species composition of biofilms, DNA was extracted from 0.1 g wet biomass using E.Z.N.A. Water DNA Kit (D5525-02, Omega Bio-tek, USA). Then primers 515F/907R were used for PCR amplification of 16S rRNA gene. The PCR product was checked with agarose gel electrophoresis and purified with E.Z.N.A.® Gel Extraction kit (Omega Bio-tek, USA). The sequencing library was generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's instructions. Successfully amplified PCR products were combined in equimolar ratios for each of the marker genes. The 16S rRNA amplicon pools were sequenced on separate Illumina MiSeq platform.

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DO and pH in the hyper-eutrophic water and sediments were determined by DO and pH meters (YSI, USA). TP in artificial hyper-eutrophic water was measured colorimetrically by the persulfate digestion-molybdophosphate reaction method. TN in artificial hyper-eutrophic water was measured by the persulphate digestion and oxidation-dual wavelength (220 nm and 275 nm) methods [32]. The dehydrogenase activity was measured using an amended triphenyltetrazolium chloride (TTC) method. Briefly, the sodium sulfide was used as reducing agent and toluene as extractant. Activity units were expressed as the amount of enzyme required to oxidize 1 mL of activated sludge suspension using 1 μg of triphenyltetrazoliumformazan (TPF) for 1 hour. TP was measured after burning the samples at 500 °C for 2 hrs by using the acid dissolution method ($HF+HNO_3$) [33].

To determine the nitrogen and phosphorus accumulation functions of the periphytic biofilms during the indoor and inter-region transplant experiments, the nitrogen and phosphorus concentrations in water were measured to calculate the TN and TP accumulation in periphytic biofilms using Eq. (1).

$$A_t = \frac{V(C_0 - C_t)}{B} \qquad (1)$$

Where A_t is TN or TP assimilated in the periphytic biofilm at time t (mg g^{-1}); C_0 is the initial TN or TP concentration in the hyper-eutrophic water (mg L^{-1}); C_t is the TN or TP concentration in the hyper-eutrophic water at time t (mg L^{-1}); V is the volume of the hyper-eutrophic water (L); B is the initial biomass of periphytic biofilm (g) and t is the accumulation time in hours. After verifying the data was normally distributed, one way ANOVA was performed using SPSS v19.0 to test the differences in carbon metabolic activities between the different phases, and nutrient accumulation by treated periphytic biofilms and the control, with a significance level of 0.05 for all analyses.

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3. Results

3.1 Carbon metabolism and dehydrogenase activity

The effects of different temperature regimes of warming (17-31 ºC), hot (31-35-31 ºC) and cooling (31-17 ºC) on a natural periphytic biofilm were compared (Fig. 1a). First, the carbon metabolic activity of the periphytic biofilm under different temperature regimes was examined (Fig. 1b). As represented by the average well color development (AWCD), there was no significant difference (p = 0.14) observed in the microbial carbon metabolic activity between the warming (AWCD = 0.22±0.07) and cooling phases (AWCD = 0.23±0.09). However, the carbon metabolic activity in the hot phase (AWCD = 0.66±0.12) was significantly higher than the other two phases (p < 0.05). In addition, the utilization of polysaccharides, phenolic acids, carboxylates, carbohydrates, and amino acids by the periphytic biofilm during the warming and cooling phases were similar, but different from that of the hot phase (Fig. 1c). The carbon metabolism of the periphytic biofilm increased in response to temperature increase but returned to the original status when temperature reverted to the initial level.

Dehydrogenase is a kind of oxidoreductases and it oxidizes a substrate by reducing an electron acceptor, usually nicotinamide adenine dinucleotide (NAD+)/ nicotinamide adenine dinucleotide phosphate (NADP+), flavin adenine dinucleotide (FAD) or flavin mononucleotide (FMN), and dehydrogenase activity directly correlates with the organic matter degradation capacity of the periphytic biofilm [34, 35]. We determined the dehydrogenase activity of the periphytic biofilms in the different temperature phases with results revealing a similar pattern to the utilization of different substrates with temperature variation (Fig. 1d). Although the temperature increase stimulated the periphytic biofilm carbon metabolic and dehydrogenase

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activities, the carbon metabolic activity and dehydrogenase activity of the periphytic biofilm reverted to the original level when temperature fell back to the initial range (17 °C).

3.2 Nitrogen and phosphorus accumulation

To investigate the nutrient regulation capabilities of the disturbed periphytic biofilm, we determined its ability to accumulate nutrients (N and P) after going through steep temperature changes and compared it to the nutrient accumulation of the undisturbed control. As shown in Figs. 1e and 1f, the accumulation of phosphorus from simulated hyper-eutrophic water was similar in both the temperature-disturbed and undisturbed periphytic biofilms. A similar trend was observed for nitrogen accumulation in both periphytic biofilms, with the two nitrogen-dynamics curves almost coincident. The nutrient accumulation capacity of the temperature disturbed periphytic biofilm was virtually unchanged.

Furthermore, the 2-year periphytic biofilm transplant experiment was conducted to elucidate whether the nutrient accumulation capability of periphytic biofilms to temperature variation was sustained in the longer term. In this experiment, the periphytic biofilm was transferred to three sites over a large transect: from Southeastern China to Southwestern China for one year and then to Northwestern China for another year (Fig. 2a). Similar to the lab experiment, the nitrogen (N) and phosphorus (P) accumulation by the control biofilm (from Nanjing) and the transplanted biofilm (in Kunming and Xi'an) were not significantly different even after being transplanted for two years (Fig. 2c&d). Both the nitrogen and phosphorus accumulation capacity of the periphytic biofilm was sustained over large temperature fluctuations.

3.3 Biofilm community composition and biodiversity

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Changes in community composition are often a response for a microbial community to external stresses such as temperature changes. In the study, the periphytic biofilm composition change in the lab experiment was investigated at genus level. A phylogenetic tree of bacteria is presented in Fig. 3. The relative abundance of identified bacteria at genus level in different phases is presented in a bar graph. Some bacteria such as *Lactococcus*, *Lysobacter* and *Merismopedia* persisted from hot to cooling phases while some microorganisms such as *Brochothrix*, *Fluviicola*, *Lysobacter* and *Tropicimonas* disappeared. The results indicate that the phyla *Bacteroidetes*, cyanobacteria, *Firmicutes*, *Planctomycetes* and *Proteobacteria* were predominant in all three phases. Moreover, cyanobacteria and *Firmicutes* were more abundant than other phyla in the periphytic biofilm, and change substantially with temperature fluctuations.

Specifically, from the warming to hot phase, the abundance of *Merismopedia* decreased, while the abundance of *Firmicutes* and *Leptolyngbya* increased greatly. In the cooling phase, the abundances of cyanobacteria and *Firmicutes* were between the warming phase and hot phase. During the warming phase, the bacterial genera were mainly *Bacillus*, *Lactococcus*, *Lysobacter*, *Merismopedia* and *Solibacillus* (Fig. 3). However, their relative abundances decreased during the subsequent phases. During the hot phase, the bacteria of the genera *Bacillus*, *Solibacillus*, *Lactococcus*, *Leptolyngbya*, *Lysobacter*, and *Merismopedia* were dominant (Fig. 3). During the cooling phase, *Lactococcus* and *Merismopedia* dominated the bacterial community (Fig. 3).

The Shannon diversity index increased from 1.91 in the warming phase to 3.40 in the hot phase, and then slightly decreased to 3.28 in the cooling phase (Fig. 4a). There was an increase in bacterial diversity in response to increasing temperature. In addition, the Simpson diversity index declined from 0.45 in the warming phase to 0.07 in the hot phase, and then increased to 0.09 in the

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cooling phase (Fig. 4a). The bacterial community therefore experienced diversity increase and then decrease in response to temperature increase and decrease. Overall, after experiencing warming, hot and cooling phases, the community composition of the periphytic biofilms changed greatly.

Furthermore, phospholipid fatty acid (PLFA) analysis was employed to compare the periphytic community structure changes between different phases, focusing on bacteria, fungi, actinomycetes, and protozoans (Fig. 4b). The PLFA content ratios of bacteria, fungi, actinomycetes, and protozoa were 34.6:10.8:0.03:1.0 for the warming phase, 19.3:4.7:0.4:1.0 for the hot phase, and 18.9:6.4:0.4:1.0 for the cooling phase. These results further demonstrated great changes in the periphytic community structure in response to temperature fluctuations. According to the PLFA contents, the biomass of bacteria, fungi, actinomycetes, and protozoa in the hot phase were all the highest and that of the warming phase were the lowest, indicating an increase in microbial biomass with temperature increase and then a decrease with the fall of temperature. Moreover, biomass and counts of bacteria and fungi were much higher than that of the actinomycetes and protozoa during all the three phases, and the PLFA content ratios between bacteria and fungi were significantly higher in the hot phase than the warming and cooling phases.

4. Discussion

Associated with the species composition change, ecological functions of the community may vary greatly [36-38]. However, in this study, the species-rich periphytic biofilm maintained the same nutrient accumulation rate under large temperature fluctuations, despite its species composition difference between the original and final communities. The higher Shannon index of hot and cooling phases than the warming phase implies the high resilience of these two phases to disturbance, verified that a high species diversity helps the community to buffer against large

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environmental variation and maintain function despite changes in specific species compositions [39, 40]. Moreover, the microbial communities from a stable environment such as a constant temperature of 25 ºC are often more sensitive and fragile to environmental changes leading to weaker acclimation than those from fluctuating environment [41].

Similar to any other species-rich communities, the species composition of periphytic biofilm undergoes dynamic changes associated with the ambient environment, such as temperature changes [7, 19]. The function of certain species can therefore be complemented by others and the collective functions of the community stay the same, referred to as functional redundancy [18, 42, 43]. In other words, the diversified species composition of periphytic biofilm guaranteed its sustainable nutrient accumulation function via functional redundancy [39, 40, 44].

The net effects of community changes on nutrient accumulation may be positive, negative, or zero, depending on the balance between sink and source processes and the habitat scales [45]. Here, the net effects on periphytic biofilms after temperature changes on their nutrient accumulation were zero, without significant differences in either nitrogen or phosphorus accumulation than the original periphytic biofilm. In addition to the functional redundancy and species composition shift, a number of fatty acids were identified during the warming, hot and cooling phases, including tetradecanoic acid, and oleic acid, which are all well known as antimicrobial compounds [46]. As a result, the biofilm community structures were potentially altered through the inter-species interactions by release of antimicrobial compounds under different temperature scenarios. More generally, stability is enhanced by limiting positive feedbacks and weakening interactions [47, 48].

Results from this study fitted to the community acclimation concept focusing on self-regulation of the community structure [41, 49]. In addition to knowing "what is inside", people

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argue that there is more intrinsic value in knowing "who does what" in a community to understand broader controls over ecosystem processes [50, 51]. Indeed, the belief that ecological functioning, such as nutrient assimilation, is affected by community structure, is supported by recent work on microbial ecosystems [51-54]. Here, the zero net effects of community changes of periphytic biofilm were confirmed for nutrient accumulation despite temperature fluctuations. Overall, the key finding from this study is that the benthic microbial community can adapt to large temperature fluctuations by regulating their functional potentials.

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Declaration of interest statement

All the authors declare that they have no competing interests.

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Figure Captions

Figure 1. Temperature regime and the activity and function of the periphytic biofilm under temperature fluctuation. (a) Imposed temperature perturbation based on the historical average annual air temperature in Nanjing, China. AHT: Average high temperature; ALT: Average low temperature; EHT: Extremely high temperature; ELT: Extremely low temperature. The dotted line represents the average temperature (26 ºC). (b) Changes in the carbon metabolic capacity of periphytic biofilms from warming, hot and cooling phases represented by Average Well Color Development (AWCD). (c) The metabolic capability of six main types of carbon sources (amine acid, amino acid, carbohydrate, carboxylic acid, phenolic acid and polymer) by the periphytic biofilms from warming, hot and cooling phases. The letters a, b denote that there is significant difference ($p < 0.05$). (d) Dehydrogenase activity of periphytic biofilms from warming, hot and cooling phases. (e) Total nitrogen (TN) and (f) total phosphorus (TP) contents in periphytic biofilms after experiencing warming, hot and cooling phases and the control cultured at a constant temperature of 25 ºC.

Figure 2. Process and results of the inter-region experiment. (a) Schematic drawing of the transplant process, from Nanjing to Kunming and then to Xi'an. (b) Changes in carbon metabolic activity represented by Average Well Color Development (AWCD) of original periphytic biofilms from Nanjing, and inter-region transplanted in Kunming and Xi'an. (c) Total nitrogen (TN) and (d) total phosphorus (TP) accumulation by original periphytic biofilms from Nanjing, and inter-region transplanted in Kunming and Xi'an.

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Figure 3. Circular maximum likelihood phylogenetic tree based on OTU of representative bacterial sequences identified in periphytic biofilms from the warming, hot, and cooling phases. The bars in the outer band represent the relative abundance of bacteria at genus level.

Figure 4. The periphytic biofilm features of the lab experiment. (a) The Shannon and Simpson indices of bacterial community in the warming, hot and cooling phases. (b) Phospholipid fatty acid (PLFA) fingerprints of bacteria, fungi, actinomycetes and protozoan in different phases. *Significant difference between warming and hot (or cooling) phases ($p < 0.05$).

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Figure 1

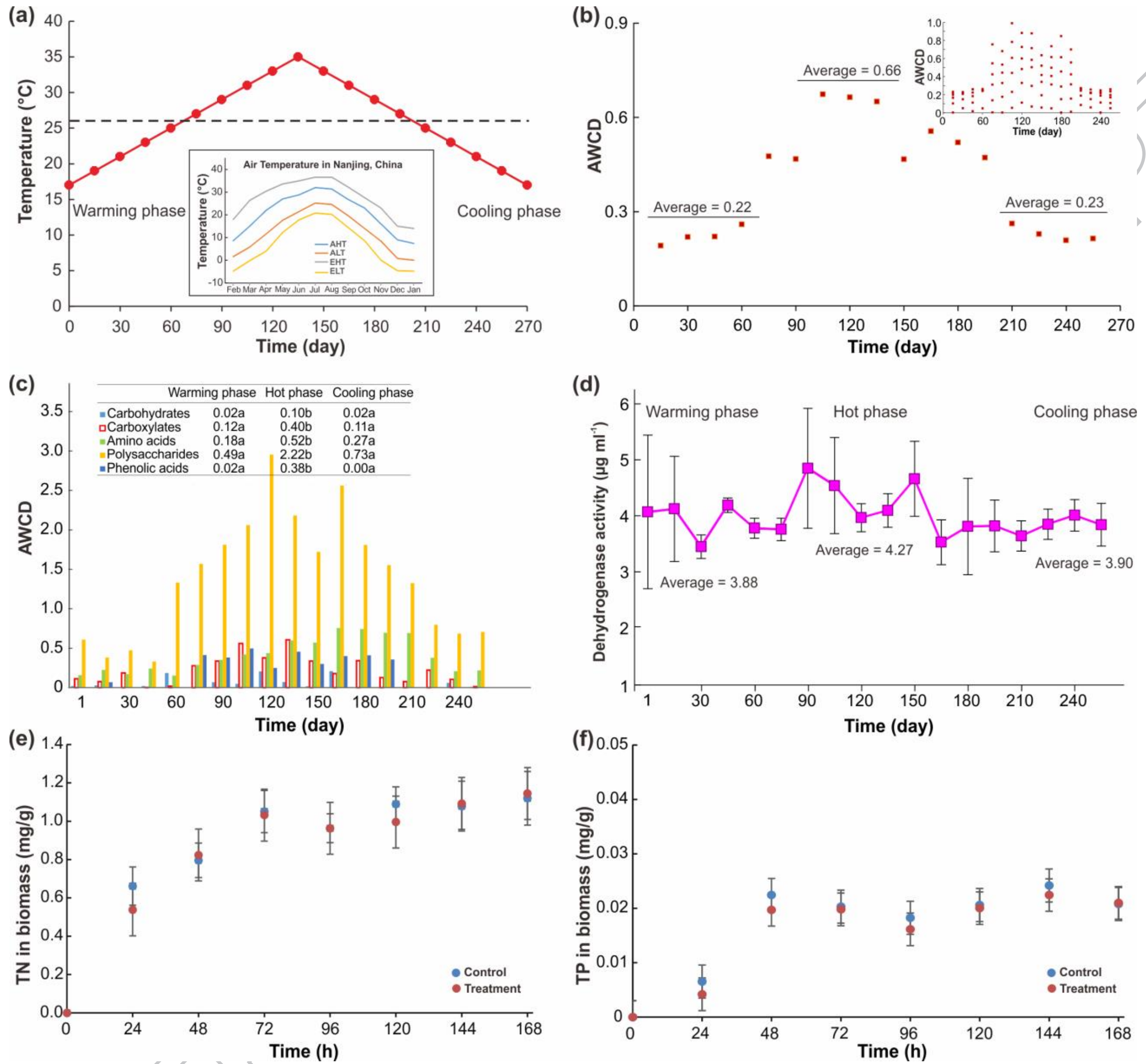

Figure 2

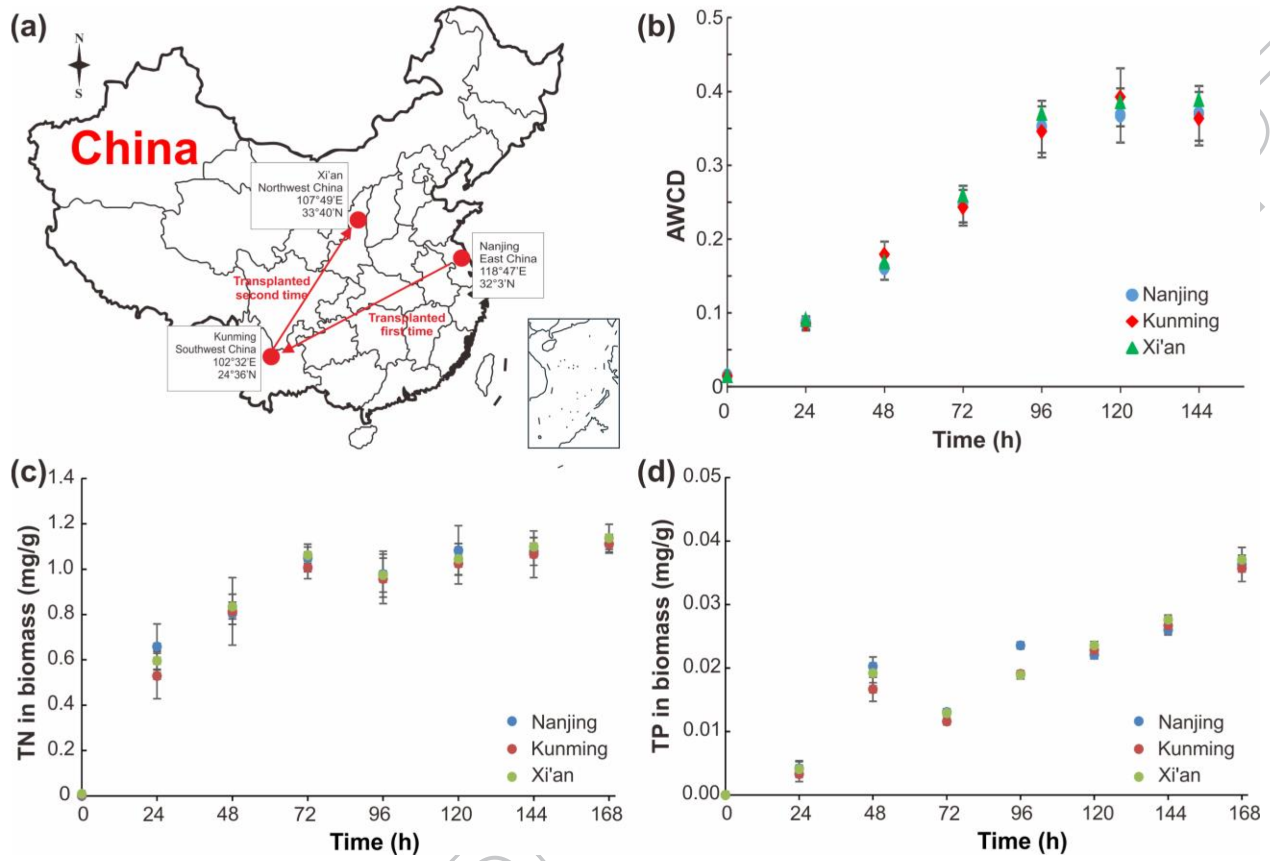

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Figure 3

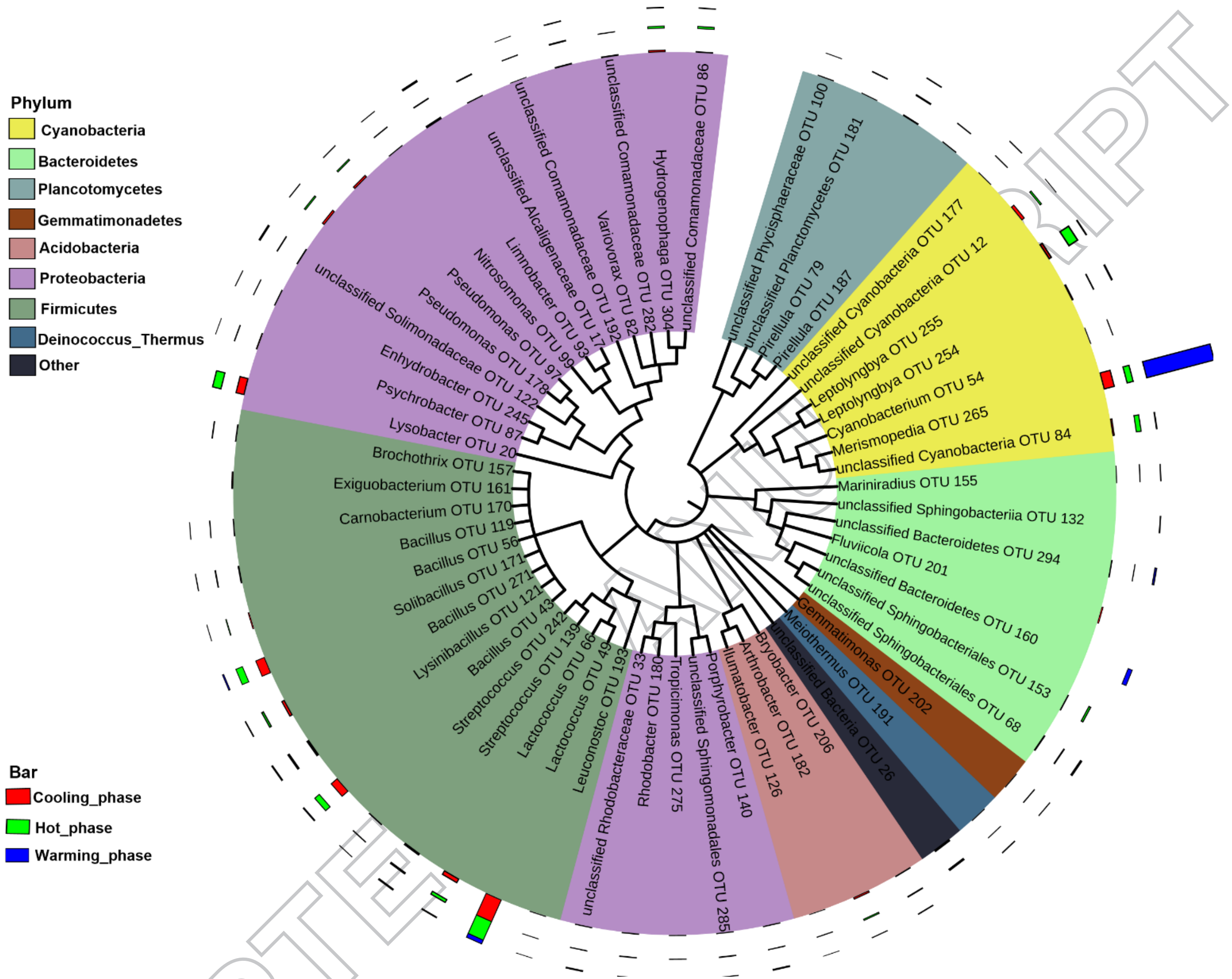

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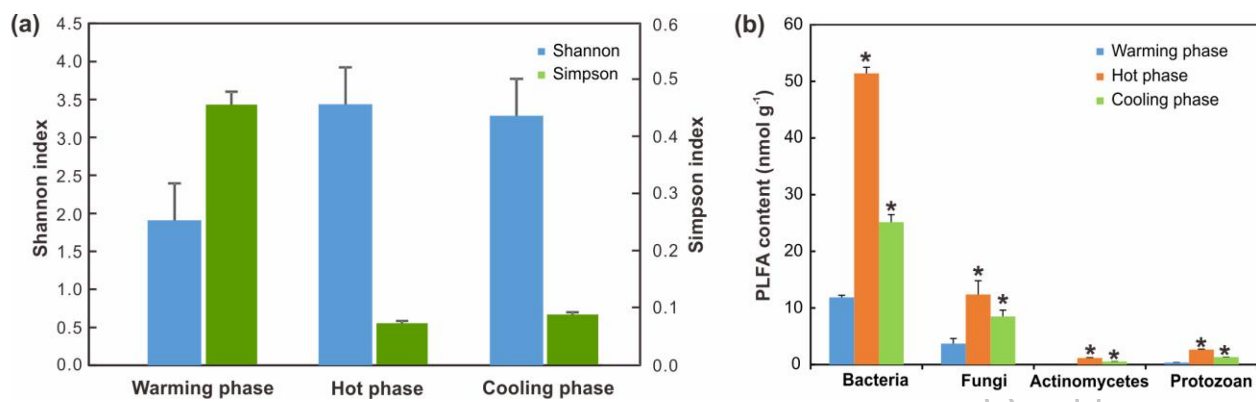
Figure 4



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