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PHOTOSYNTHETIC ADAPTATION TO LIGHT AVAILABILITY SHAPES THE ECOLOGICAL SUCCESS OF BLOOM-FORMING CYANOBACTERIUM *PSEUDANABAENA* TO IRON LIMITATION[1]

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The poorly understood filamentous cyanobacterium *Pseudanabaena* is commonly epiphytic on *Microcystis* colonies and their abundances are often highly correlated during blooms. The response and adaptation of *Microcystis* to iron limitation have been extensively studied, but the strategies *Pseudanabaena* uses to respond to iron limitation are largely unknown. Here, physiological responses to iron limitation were compared between one *Pseudanabaena* and two *Microcystis* strains grown under different light intensities. The results showed that low-intensity light exacerbated, but high-intensity light alleviated, the negative effect of iron limitation on *Pseudanabaena* growth relative to two *Microcystis* strains. It was found that robust light-harvesting and photosynthetic efficiency allowed adaptation of *Pseudanabaena* to low light availability relative to two *Microcystis* strains only during iron sufficiency. The results also indicated that a larger investment in the photosynthetic antenna probably contributed to light/iron co-limitation of *Pseudanabaena* relative to two *Microcystis* strains under both light and iron limitation. Furthermore, the lower antenna pigments/chlorophyll *a* ratio and photosynthetic efficiency, and higher nonphotochemical quenching and saturation irradiance provided *Pseudanabaena* photoadaptation and photoprotection advantages over the two *Microcystis* strains under the high-light condition. The lower investment in antenna pigments of *Pseudanabaena* than the two *Microcystis* strains under high-light intensity is likely an efficient strategy for both saving iron quotas and decreasing photosensitivity. Therefore, when compared with *Microcystis*, the high plasticity of antenna pigments, along with the excellent photoadaptation and photoprotection ability of *Pseudanabaena*, probably ensures its ecological success under iron limitation when light is sufficient.

Key index words: **blooms; iron limitation; light; *Microcystis*; photosynthesis; *Pseudanabaena***

***Abbreviations*: APC, allophycocyanin; CAR, carotenoids; I_k, saturation irradiance; NPQ, nonphotochemical quenching; PC, phycocyanin; PE, phycoery-**

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thrin; $rETR_{max}$, the maximal relative electron transport rate; TPP/Chl *a*, total phycobilin pigments to Chl *a* ratio; α, photosynthetic efficiency; *μ*, the specific growth rate

Pseudanabaena is a small, filamentous, and epiphytic cyanobacterial species commonly embedded within or attached to the sheath of colonies of *Microcystis*, which is a bloom-forming species (Chang 1985, Pankow 1986, Patterson and Wilson 1995, Mayer et al. 1997, Vasconcelos and Pereira 2001, Yarmoshenko et al. 2013, Zhuang et al. 2015, Agha et al. 2016). The abundance of these two species is highly correlated during blooms (Ilhe 2008, Berry et al. 2017), but most studies focus on *Microcystis* because of its high abundance and toxin production (de Figueiredo et al. 2004). *Microcystis* cells are enveloped by a mucilaginous sheath and aggregate together to form large colonies that can produce dense scum on the water surface (Holt et al. 1994). It has been suggested that the formation of *Microcystis* blooms is regulated by both light and iron (Fe) availability in freshwater ecosystems (Aizaki and Aoyama 1995, Visser et al. 1996, Havens et al. 1998, Imai et al. 1999, Nagai et al. 2004, Xu et al. 2013a). However, it is still unclear how light intensity regulates the physiological responses of *Pseudanabaena* under iron limitation to achieve its ecological success in freshwater ecosystems.

Iron is an essential micronutrient for photosynthetic organisms as it is required for photosynthesis, respiration, nitrogen fixation, and the biosynthesis of photosynthetic pigments (chlorophyll *a*, Chl *a*; carotenoid, CAR; phycobilins; Miller et al. 1984, Geider and La Roche 1994, Kudo and Harrison 1997, Bouvier et al. 1998, Behrenfeld and Milligan 2013). It has been demonstrated that the growth of phytoplankton is limited by iron limitation in many lakes, where iron bioavailability can be as low as in the ocean (Imai et al. 1999, Twiss et al. 2000, McKay et al. 2004, Sterner et al. 2004, Nagai et al. 2007, North et al. 2007, 2008, Xu et al. 2013a). Iron limitation has been shown to reduce photosynthetic activity, photosynthetic pigment content, and nonphotochemical quenching (NPQ) of *Microcystis* and *Pseudanabaena* (Li et al. 2016, 2017, Xu et al. 2017). Xu et al. (2017) found that low iron concentration (100 nM) had no significant effect on the growth of *Pseudanabaena* but caused slower growth of both *Microcystis aeruginosa* FACHB-912 and *Microcystis flos-aquae* FACHB-1028. Cyanobacteria have evolved several strategies to respond to iron limitation including upregulating expression of iron uptake genes (Kranzler et al. 2011, Jiang et al. 2015), decreasing iron demand (Peers and Price 2006), and improving iron utilization (Noinaj et al. 2010). We recently demonstrated an iron adaptation mechanism of colonial *Microcystis*, where capsular polysaccharides can be used as an iron reservoir to help *Microcystis* cells cope with fluctuating iron conditions in neutral and slightly alkaline environments (Li et al. 2016). However, mechanisms used by *Pseudanabaena* to overcome iron limitation are poorly understood.

Microcystis can tolerate high-light conditions with little photoinhibition (Reynolds 1993, Huisman et al. 2004). Accordingly, *Microcystis* has evolved effective strategies, such as decreased light harvesting by decreasing Chl *a* and phycocyanin (PC) content (Raps et al. 1983, 1985), enhanced photoprotection by increasing the CAR/Chl *a* ratio (Raps et al. 1983, Deblois and Juneau 2010), and efficient dissipation of excess excitation energy by NPQ (Deblois and Juneau 2010, Zhang et al. 2011). However, there is very little evidence on how *Pseudanabaena* regulates its light harvesting and photoprotection to adapt to high-light conditions.

The massive accumulation of buoyant cyanobacterial species like *Microcystis* reduces light penetration at the water surface (Jacoby et al. 2000, Huisman et al. 2004). In addition to diurnal and seasonal variation in solar radiation (Bishop and Rossow 1991), turbulent mixing of the water column (Lavaud et al. 2007), dissolved organic matter suspended in the water column (Klug 2002), and the vertical distribution of cyanobacterial cells (Zhang et al. 2008) alter light availability in aquatic ecosystems. Low light availability could provide advantages for low light-adapted species with high concentrations of light-harvesting pigments, especially species with additional phycoerythrin (PE; Scheffer et al. 1997, Samsonoff and MacColl 2001). *Microcystis* exhibits high values of light-saturated oxygen evolution rate, Chl *a* and PC content, and photosynthetic efficiency (α) for higher light harvesting under low light availability (Zevenboom and Mur 1984, Bañares-España et al. 2013). PC- and PE-enriched *Pseudanabaena* strains display extensive complementary chromatic adaptation (Acinas et al. 2009), which facilitates their competitiveness over both green and red *Synechococcus* strains in fluctuating light quality environments (Stomp et al. 2008). This indicates that modification of light-harvesting pigments plays an essential role in the success of *Pseudanabaena* under fluctuating light quality environments. However, it is still unclear how the light-harvesting pigments of *Pseudanabaena* are modified to adapt to different light availabilities.

Iron requirements increase as light intensity decreases, likely driven by additional light-harvesting pigments and Fe-containing redox proteins needed in photosynthesis (Sunda and Huntsman 1997, Cloern 1999). Conversely, reduced investment in light harvesting and photosynthetic machinery under high-light conditions can reduce iron demand while growth rate increases. The buoyancy of *Pseudanabaena* is too weak to float itself to the water surface and achieve frequent high light exposure but epiphytic growth with floating *Microcystis* colonies can

increase mean light availability for *Pseudanabaena* (Damerval et al. 1991, Acinas et al. 2009). Quantifying the response of *Pseudanabaena* and *Microcystis* species to iron availability under different light conditions will help to explain the ecological success strategies of cyanobacterial blooming species in freshwater ecosystems.

To better understand the physiological strategies of these important cyanobacteria to iron limitation, one *Pseudanabaena* and two *Microcystis* species isolated from Lake Taihu (the third largest freshwater lake in China) were cultured under four light and iron conditions: low-light and iron-limited, low-light and iron-replete, high-light and iron-limited, and high-light and iron-replete. We compared the growth rate and cell size between these species under different conditions to characterize how they respond to iron limitation under different light intensities. We further compared photosynthetic pigments, photosynthetic capacity, and NPQ between these species under different conditions to determine how they coordinate their light harvesting, photoprotection, and iron demand to adapt to iron limitation under different light intensities.

MATERIALS AND METHODS

Strains and culture conditions. *Microcystis aeruginosa* FACHB-912 (*Microcystis* 912), *Microcystis flos-aque* FACHB-1028 (*Microcystis* 1028), and *Pseudanabaena* sp. FACHB-1282 (*Pseudanabaena*) used in this study were isolated from Lake Taihu and obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB), the Chinese Academy of Sciences. *Microcystis aeruginosa* and *Microcystis flos-aque* are two representative bloom-forming cyanobacterial species in Lake Taihu. *Microcystis* FACHB-912 and FACHB-1028 are unicellular while *Pseudanabaena* FACHB-1282 grew in short trichomes in our laboratory culture conditions. Cells were grown in Fraquil, a medium designed for studying the trace-metal physiology of phytoplankton (Morel et al. 1975), and were maintained under two iron conditions. The iron-limited condition contained 100 nM $FeCl_3$ for *Microcystis* 912, and 50 nM $FeCl_3$ for *Microcystis* 1028 and *Pseudanabaena*, according to our former study on the different iron responses of these three species (Xu et al. 2017). The iron-replete condition contained 1,000 nM $FeCl_3$ for all three species. Iron speciation was manipulated by the addition of variable total concentrations of iron and EDTA, and free iron ion concentrations were calculated using Visual MINTEQ Version 3.0 (Gustafsson JP. http://www.lwr.kth.se/english/OurSoftware/vminteq/). The addition of 50 nM, 100 nM (iron-limited: −Fe), and 1000 nM (iron-replete: +Fe) $FeCl_3$ to Fraquil medium resulted in free ferric iron concentrations of pFe = 21.6 (pFe = $-\log [Fe^{3+}]$), pFe = 21.3 and pFe = 20.3, respectively. Free concentrations of all other trace metals and the pH values of cultures were kept constant. Cyanobacteria were grown at 25°C on a 14:10 h light–dark cycle and shaken three times per day. Irradiance was provided by cool-white fluorescence lamps from the side of the bottles at two light intensities of 12 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (low-light: LL) and 90 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (high-light: HL), measured with a spherical micro quantum sensor (US-SQS/WB, Walz GmbH, Effeltrich, Germany). Prior to experimentation, cyanobacterial cells were adapted to all treatments (iron concentrations and light intensities) for at least 15 generations, and then maintained in exponential phase by serial dilutions. Stock and experimental cultures (50 mL) were maintained in 125 mL Nalgene polycarbonate bottles (Nalge Nunc International Corporation, Rochester, NY, USA) because they do not significantly absorb or leach trace metals (Price et al. 1989). All plasticware in contact with the medium were soaked overnight in 1 N HCl, rinsed with trace metal grade 1 N HCl, and finally rinsed five times with Milli-Q water (18.2 MΩ $\cdot$ cm^{-1}). Cell density and size were determined by using a Multisizer™ 3 Coulter Counter® (Beckman Coulter Inc., Brea, CA, USA).

The specific growth rate (μ) was calculated according to the formula $\mu = (\ln N_1 - \ln N_0)/d$, where N_0 and N_1 are cell number at the beginning and the end of the exponential growth, and d is the duration of the growth period in days.

Chlorophyll fluorescence measurements. Cultures in exponential phase were dark-acclimated for 15 min before the measurement of light response curves using a WATER-PAM Chlorophyll Fluorometer (Walz GmbH, Effeltrich, Germany) following Xu et al. (2013b), with the following modifications. The actinic light intensity was automatically increased from 71 to 1098 μmol photons $\cdot m^{-2} \cdot s^{-1}$ in eight successive steps. Cells were kept for 2 min at each actinic light intensity before taking measurements. The fluorescence yields F' and F_m' were respectively determined before or during the saturating light pulse (800 ms, 3,000 μmol photons $\cdot m^{-2} \cdot s^{-1}$) triggered at the end of each illumination period. The Genty parameter YIELD was calculated as follows: YIELD = $(F_m' - F)/F_m'$ (Genty et al. 1989). The relative electron transport rate (rETR) at a given actinic irradiance was calculated by the formula: rETR = 0.5 × 0.84 × YIELD × PAR, where PAR is the actinic irradiance in μmol photons $\cdot m^{-2} \cdot s^{-1}$ (Ralph et al. 2002). The maximal relative electron transport rate ($rETR_{max}$) and photosynthetic efficiency (α) were calculated by fitting the rapid light response curve to an exponential function modified from Jassby and Platt (1976): $rETR = rETR_{max} \times [1 - \exp(-\alpha \times I/rETR_{max})]$, where rETR represents relative electron transport rate, and I is irradiance. The saturation irradiance (I_k) was calculated by $I_k = rETR_{max}/\alpha$.

The fluorescence induction curve was measured for exponentially growing cultures using a WATER-PAM Fluorometer after 15 min of dark acclimation. The actinic light intensities used in the fluorescence induction curve were 24 and 94 μmol photons $\cdot m^{-2} \cdot s^{-1}$ for samples grown under low-light and high-light conditions, respectively. Simultaneously, saturating flashes (800 ms, 3,000 μmol photons $\cdot m^{-2} \cdot s^{-1}$) were given periodically (every 40 s) to obtain the maximal fluorescence yield for a light-adapted sample (F_m'). The maximal fluorescence yield (F_{mDCMU}) was obtained by adding 20 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) at the end of measurement according to Campbell et al. (1998). NPQ was calculated according to the following equation: $NPQ = (F_{mDCMU} - F_m')/F_m'$ (Campbell et al. 1998).

Pigment measurements. The Chl *a* and CAR contents were measured and calculated according to Lichtenthaler and Buschmann (2001). Cell density was initially counted with a 1 mL sub-sample as above. Cells were filtered on a membrane filter (1 μm pore size, 25 mm diameter), re-suspended in 95% ethanol, and incubated for 24 h in the dark at 4°C. The extract solutions were centrifuged at 7,500*g* for 10 min, and the absorbance values of the supernatants at 664.1 nm, 648.6 nm, and 470.0 nm were measured with a Cary 300 Bio UV-Visible spectrophotometer (Varian, Inc., Palo Alto, CA, USA). For the measurements of phycocyanin (PC), allophycocyanin (APC), and phycoerythrin (PE) contents, samples were

resuspended in 3.5 mL of 0.1 M phosphate buffer (mixing equal volumes of 0.1 mol · L^{-1} KH_2PO_4 with 0.1 mol · L^{-1} K_2HPO_4 solutions, pH 6.8), and then frozen in liquid nitrogen and thawed at 20°C six times. The extract solutions were centrifuged at 7,500*g* for 10 min, and the absorbance values of the supernatants were determined at 565 nm, 615 nm, and 650 nm. The concentrations of PC, APC, and PE were calculated according to Lüder et al. (2001).

Statistical analyses. All experiments were performed using four independent biological replicates. All data are presented as mean ± standard deviation (SD) or mean ± standard error (SE). Statistical analyses were carried out using R version 3.4.2 according to Zhang et al. (2015). A two-way ANOVA was used to determine the main effect of light, iron, and their interactions for these variables. A one-way ANOVA and Tukey's honestly significant difference (HSD) test were conducted to test the differences among treatments. Normality of residuals was tested with a Shapiro–Wilk's test. Levene's test was conducted graphically to test for homogeneity of variances in case of significant data. A generalized least squares (GLS) model was used to stabilize heterogeneity if variances were heterogeneous.

RESULTS

The effect of iron limitation on the growth of Pseudanabaena *under low-light versus high-light.* The growth rate of all three species was reduced under iron limitation for both light intensities, but the effect was not as strong on cell volume (Fig. 1). High-light conditions induced an increase in both the growth rate and cell volume of all species under both iron conditions except for *Microcystis* 912 under iron-limited conditions (ANOVA, All $F_{3,12} > 34$, $P < 0.0001$; Tukey's HSD, $P < 0.05$). Iron and light intensities affected the growth rates and cell volume of all species and there was an interaction between iron conditions and light intensities (Table S1 in the Supporting Information). Under the four light and iron combinations, *Pseudanabaena* had a highly variable growth rate, whereas *Microcystis* 912 had highly variable cell volume. The growth rate of *Pseudanabaena* was reduced more significantly by iron limitation under low-light conditions than under high-light conditions when compared with that of the two *Microcystis* strains (ANOVA, All $F_{2,9} > 15$, $P < 0.0001$; Tukey's HSD, $P < 0.01$). The growth rate of *Pseudanabaena* increased more than that of the two *Microcystis* strains under high-light, especially under iron-limited conditions (ANOVA, $F_{2,9} = 41$, $P < 0.0001$; Tukey's HSD, $P < 0.0001$). The cell volume of *Microcystis* 1028 decreased with iron limitation under both light intensities (ANOVA, $F_{3,12} = 145$, $P < 0.0001$; Tukey's HSD, $P < 0.001$), while iron limitation induced increases in cell volume for both *Microcystis* 912 and *Pseudanabaena* under the low-light condition (ANOVA, All $F_{3,12} > 481$, $P < 0.0001$; Tukey's HSD, $P < 0.01$), with no effect under the high-light condition. The cell volume of *Microcystis* 912 increased more than that of *Microcystis* 1028 and *Pseudanabaena* under high light for both iron conditions (ANOVA, All $F_{2,9} > 198$, $P < 0.0001$; Tukey's HSD, $P < 0.0001$).

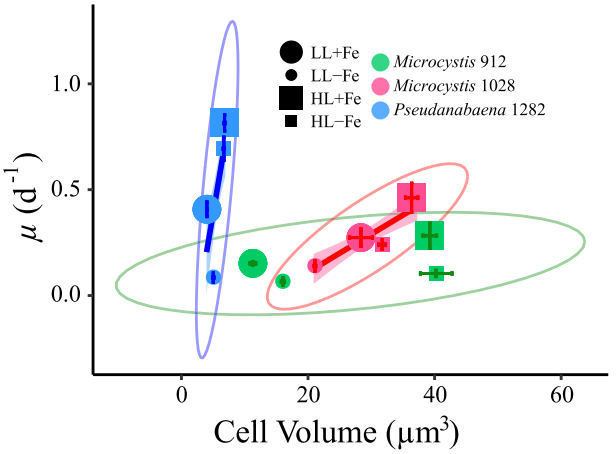

FIG. 1. Growth rate as a function of cell volume of *Microcystis* 912, *Microcystis* 1028, and *Pseudanabaena* 1282 grown under LL + Fe (big-circles symbol, low-light, and iron-replete), LL − Fe (small-circles symbol, low-light, and iron-limited), HL + Fe (big square symbol, high-light, and iron-replete) and HL − Fe (small square symbol, high-light, and iron-limited) conditions. Green, red, and blue colors represent *Microcystis* 912, *Microcystis* 1028, and *Pseudanabaena* 1282, respectively. Linear regressions with $P < 0.05$ are plotted in the figure. Shadings represent 95% confidence limits of the regressions. Ellipses represent the 95% confidence interval around the mean to represent the different effect of treatments on different species. Averages (±SD; n = 4) for each species and each condition are plotted. Least squares linear regression results are provided in Table S2 and Tukey's HSD comparing slopes between species in Table S3.

Robust photosynthetic capability contributes to faster growth rate of Pseudanabaena. Photosynthetic parameters, $rETR_{max}$, α, I_k, and NPQ, of all three species were significantly reduced by iron limitation under low-light (Table 1; ANOVA, All $F_{3,12} > 18$, $P < 0.0001$; Tukey's HSD, $P < 0.05$), with little or no effect under high-light conditions. High-light conditions caused an increase in I_k and NPQ for all species irrespective of iron conditions (except for *Microcystis* 1028 under iron-replete conditions), an increase in $rETR_{max}$ under iron limitation, and a reduction in α under iron-replete conditions (ANOVA, All $F_{3,12} > 18$, $P < 0.0001$; Tukey's HSD, $P < 0.05$). The growth rate increased linearly with $rETR_{max}$ with similar slopes for all three species (Fig. 2A; Tables S2, S3 in the Supporting Information). However, *Pseudanabaena* exhibited much higher $rETR_{max}$ values than the two *Microcystis* strains under iron-replete treatments (Table 1; ANOVA, All $F_{2,9} > 10$, $P < 0.01$; Tukey's HSD, $P < 0.01$). The growth rate increased linearly with both the saturation irradiance (I_k) and NPQ with similar slopes for all three species (Fig. 2, C and D; Tables S2, S3). However, *Pseudanabaena* exhibited much higher I_k values than the two *Microcystis* strains under all four treatment conditions (Table 1; ANOVA, All $F_{2,9} > 17$, $P < 0.001$; Tukey's HSD, $P < 0.05$). *Pseudanabaena* also exhibited higher NPQ values than the two *Microcystis* strains for all four

TABLE 1. Effects of light and iron availability on chlorophyll fluorescence parameters and pigment ratios of *Microcystis* 912, *Microcystis* 1028, and *Pseudanabaena* 1282.

Species	Conditions	$rETR_{max}$	α	I_k	NPQ	CAR/Chl *a*	PC/Chl *a*	APC/Chl *a*	PE/Chl *a*	TPP/Chl *a*
Microcystis 912	LL + Fe	62.1 ± 7.6^{a}	0.17 ± 0.01^{a}	354.2 ± 24.3^{a}	0.14 ± 0.01^{a}	0.5 ± 0.1^{a}	4.8 ± 0.7^{a}	4.1 ± 0.5^{a}	—	8.9 ± 0.9^{a}
	LL − Fe	36.6 ± 4.6^{b}	0.13 ± 0.01^{b}	272.6 ± 17.5^{b}	0.05 ± 0.01^{b}	0.9 ± 0.1^{b}	8.4 ± 1.9^{b}	12.6 ± 2.5^{b}	—	21.0 ± 0.9^{b}
	HL + Fe	68.6 ± 7.1^{a}	0.14 ± 0.01^{b}	503.3 ± 24.0^{c}	0.22 ± 0.02^{c}	1.2 ± 0.1^{c}	5.9 ± 0.6^{a}	6.1 ± 0.8^{a}	—	12.0 ± 1.3^{c}
	HL − Fe	58.2 ± 2.8^{a}	0.12 ± 0.01^{b}	476.1 ± 29.4^{c}	0.10 ± 0.01^{d}	1.1 ± 0.1^{bc}	5.5 ± 0.2^{a}	4.0 ± 0.7^{a}	—	9.5 ± 0.6^{a}
Microcystis 1028	LL + Fe	67.1 ± 2.6^{a}	0.16 ± 0.01^{a}	410.2 ± 23.6^{a}	0.24 ± 0.01^{a}	0.7 ± 0.0^{a}	5.0 ± 0.9^{a}	7.5 ± 1.1^{a}	—	12.6 ± 0.4^{a}
	LL − Fe	27.1 ± 1.3^{b}	0.11 ± 0.01^{b}	254.2 ± 15.2^{b}	0.03 ± 0.01^{b}	1.2 ± 0.1^{b}	5.0 ± 0.2^{a}	6.8 ± 1.2^{a}	—	11.9 ± 1.0^{a}
	HL + Fe	68.0 ± 5.9^{a}	0.14 ± 0.02^{c}	495.5 ± 55.3^{c}	0.20 ± 0.01^{c}	1.0 ± 0.1^{c}	3.0 ± 0.1^{b}	4.6 ± 0.8^{b}	—	7.5 ± 0.2^{b}
	HL − Fe	54.3 ± 2.7^{c}	0.14 ± 0.01^{c}	402.4 ± 38.6^{a}	0.09 ± 0.01^{d}	1.5 ± 0.1^{d}	6.3 ± 0.7^{c}	7.9 ± 1.3^{a}	—	14.2 ± 0.7^{c}
Pseudanabaena 1282	LL + Fe	91.4 ± 3.4^{a}	0.20 ± 0.01^{a}	461.8 ± 29.9^{a}	0.40 ± 0.02^{a}	0.4 ± 0.0^{a}	1.2 ± 0.2^{a}	1.2 ± 0.1^{a}	1.8 ± 0.1^{a}	4.2 ± 0.0^{a}
	LL − Fe	36.1 ± 4.4^{b}	0.11 ± 0.02^{b}	342.7 ± 24.3^{b}	0.15 ± 0.03^{b}	0.9 ± 0.1^{b}	2.9 ± 0.1^{b}	2.7 ± 0.1^{b}	3.5 ± 0.0^{b}	9.1 ± 0.2^{b}
	HL + Fe	89.2 ± 9.3^{a}	0.09 ± 0.01^{bc}	1036.6 ± 86.0^{c}	0.53 ± 0.01^{c}	0.6 ± 0.0^{c}	0.7 ± 0.1^{c}	0.8 ± 0.1^{c}	0.6 ± 0.0^{c}	2.0 ± 0.1^{c}
	HL − Fe	58.6 ± 3.8^{c}	0.08 ± 0.01^{c}	760.6 ± 51.8^{d}	0.23 ± 0.03^{d}	0.6 ± 0.0^{c}	0.4 ± 0.1^{d}	0.7 ± 0.1^{c}	0.6 ± 0.1^{c}	1.7 ± 0.1^{d}

LL + Fe (low-light and iron-replete conditions), LL − Fe (low-light and iron-limited conditions), HL + Fe (high-light and iron-replete conditions), HL − Fe (high-light and iron-limited conditions). [a, b, c, d] Values with different superscript letters for each species in the same column are significantly different (Tukey's HSD, $P < 0.05$). Data are mean ± SD (n = 4).

$rETR_{max}$, the maximal relative electron transport rate; α, photosynthetic efficiency; I_k, saturation irradiance; NPQ, nonphotochemical quenching; CAR/Chl *a*, carotenoids/chlorophyll *a* ratio; PC/Chl *a*, phycocyanin/ chlorophyll *a* ratio; APC/Chl *a*, allophycocyanin/chlorophyll *a* ratio; TPP/Chl *a*, total phycobilin pigments/chlorophyll *a* ratio.

treatments (Table 2; ANOVA, All $F_{2,9} > 37$, $P < 0.0001$; Tukey's HSD, $P < 0.001$). No significant relationships between the growth rate and the photosynthetic efficiency (α) were observed for all three species (Fig. 2B; Table S3). However, *Pseudanabaena* exhibited the lowest α values induced by high-light condition regardless of the iron conditions, and the highest α value under LL + Fe conditions (Table 1; ANOVA, All $F_{2,9} > 21$, $P < 0.001$; Tukey's HSD, $P < 0.01$). The growth rate increased with the increasing cellular CAR content for all three species with similar slopes in the two *Microcystis* strains and a higher slope in *Pseudanabaena* (Fig. 2E; Tables S2, S3; ANOVA, $F_{2,42} = 28$, $P < 0.01$). The growth rate decreased with increasing TPP/Chl *a* in *Microcystis* 1028 (Fig. 2F; Table S2), with a lower slope for *Pseudanabaena* (Table S3; ANOVA, $F_{1,28} = 2.3$, $P = 0.1$), but not *Microcystis* 912.

Photosynthetic pigments and TPP/Chl a regulate the photoadaptation of Pseudanabaena. Under the low-light condition, iron limitation was associated with a decrease in all photosynthetic pigments in *Microcystis* 912 by 20.2%–66.0% (except for APC), in *Microcystis* 1028 by 49.0%–75.9%, and in *Pseudanabaena* by 27.8%–69.9% (Table 2; ANOVA, All $F_{3,12} > 8$, $P < 0.01$; Tukey's HSD, $P < 0.01$). Under the high-light condition, iron limitation had no effect on the pigment content of *Microcystis* 912, but resulted in decreased pigment content in *Microcystis* 1028 by 21.7%–58.7% and by 12.5%–55.9% in *Pseudanabaena* (ANOVA, All $F_{3,12} > 30$, $P < 0.0001$; Tukey's HSD, $P < 0.05$). Under iron-replete conditions, the high-light condition caused a decrease in Chl *a* in *Microcystis* 912 and *Pseudanabaena* by 43.6% and 32.1%, respectively (ANOVA, All $F_{3,12} > 65$, $P < 0.0001$; Tukey's HSD, $P < 0.01$). In contrast, under iron-limited conditions, the high-light treatment resulted in an increase in Chl *a* of *Microcystis* 912, *Microcystis* 1028, and *Pseudanabaena* by 80.9%, 52.6%, and 73.2%, respectively (ANOVA, All $F_{3,12} > 65$, $P < 0.0001$; Tukey's HSD, $P < 0.05$). The decrease in TPP content under the high-light condition for *Pseudanabaena* was larger than for the two *Microcystis* strains, irrespective of the iron concentration (ANOVA, All $F_{2,9} > 267$, $P < 0.0001$; Tukey's HSD, $P < 0.0001$), but high-light induced an increase in TPP content in *Microcystis* 1028 under the iron-limited condition (ANOVA, All $F_{3,12} > 767$, $P < 0.0001$; Tukey's HSD, $P < 0.0001$).

The TPP/Chl *a* values were lower for *Pseudanabaena* than for the two *Microcystis* strains for all treatments (Table 1; ANOVA, All $F_{2,9} > 167$, $P < 0.0001$; Tukey's HSD, $P < 0.01$). Under the low-light condition, iron limitation caused TPP/Chl *a* values to increase in *Microcystis* 912 and *Pseudanabaena* by 73.9%–207.1% and 100.3%–139.3%, respectively (ANOVA, All $F_{3,12} > 9.4$, $P < 0.01$; Tukey's HSD, $P < 0.01$). In contrast, under high-light conditions, iron limitation resulted in the decrease in the TPP/Chl *a* in *Microcystis* 912 by

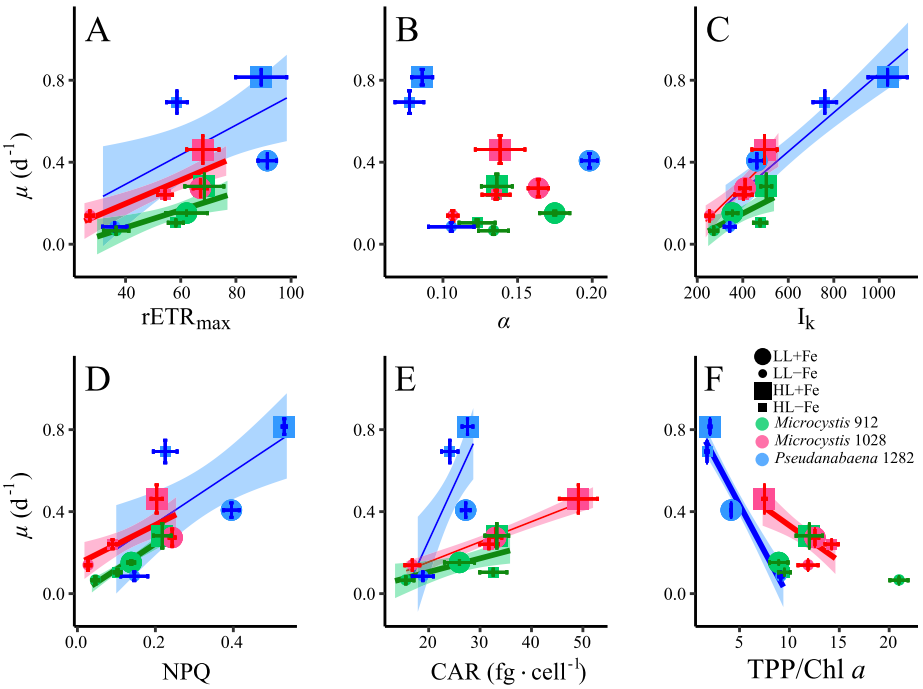

FIG. 2. Growth rate as a function of $rETR_{max}$ (the maximal relative electron transport rate) (A), α (photosynthetic efficiency) (B), I_k (saturation irradiance) (C), NPQ (nonphotochemical quenching) (D), CAR (carotenoids) (E) and TPP/Chl *a* (total phycobilin pigments/chlorophyll *a* ratio) (F) for *Microcystis* 912, *Microcystis* 1028, and *Pseudanabaena* 1282 grown under LL + Fe (big-circles symbol, low-light, and iron-replete), LL − Fe (small-circles symbol, low-light, and iron-limited), HL + Fe (big square symbol, high-light, and iron-replete) and HL − Fe (small square symbol, high-light, and iron-limited) conditions. Green, red, and blue colors represent *Microcystis* 912, *Microcystis* 1028, and *Pseudanabaena* 1282, respectively. Linear regressions with $P < 0.05$ are plotted in the figures. Shadings represent 95% confidence limits of the regressions. Averages (±SD; n = 4) for each species and each condition are plotted. Least squares linear regression results are provided in Table S2 and Tukey's HSD comparing slopes between species in Table S3.

20.8% (ANOVA, $F_{3,12} = 132$, $P < 0.0001$; Tukey's HSD, $P < 0.05$), and the PC/Chl *a* in *Pseudanabaena* by 42.7% (ANOVA, $F_{3,12} = 418$, $P < 0.0001$; Tukey's HSD, $P < 0.01$). However, iron limitation had no effect on these ratios in *Microcystis* 1028 under the low-light condition but prompted the increase in these ratios under the high-light condition. The decrease in TPP/Chl *a* was larger in *Pseudanabaena* when compared with the two *Microcystis* strains under the high-light condition irrespective of iron concentrations (ANOVA, All $F_{2,9} > 167$, $P < 0.0001$; Tukey's HSD, $P < 0.0001$).

Small-sized Pseudanabaena *possessed higher cellular pigment concentration than two* Microcystis *strains.* The small-sized *Pseudanabaena* possessed higher cellular pigment concentration than the two larger-sized *Microcystis* strains for all treatments except for HL − Fe, where *Pseudanabaena* and *Microcystis* 1028 had equal TPP contents (Table 2; ANOVA, All $F_{2,9} > 29$, $P < 0.001$; Tukey's HSD, $P < 0.001$). For all three species, cellular pigment concentrations decreased with increasing cell volume (Fig. 3; Tables S2 and S3). High-light conditions alleviated the effect of iron limitation on the cellular pigment concentration of *Microcystis* 912 and *Pseudanabaena* more than that of *Microcystis* 1028. Under iron-replete conditions, high-light conditions caused a reduction in cellular pigment concentration in both *Microcystis* 912 and *Pseudanabaena*, but caused a limited reduction or increase in *Microcystis* 1028 (ANOVA, All $F_{3,12} > 44$, $P < 0.0001$; Tukey's HSD, $P < 0.05$). Under iron-limited conditions, high-light conditions caused an increase in the cellular CAR concentration only in *Microcystis* 1028, and caused similar TPP concentration decreases in *Microcystis* 912 and *Pseudanabaena* (ANOVA, $F_{3,12} = 44$, $P < 0.0001$; Tukey's HSD, $P < 0.01$).

Pseudanabaena *could better balance light harvesting and photoprotection than two* Microcystis *strains.* NPQ increased with increasing cellular CAR content in all three species (Fig. 4A), but with a much higher slope in *Pseudanabaena* than in both *Microcystis* strains (Tables S2 and S3; ANOVA, $F_{2,42} = 9.4$, $P < 0.0001$). TPP/Chl *a* decreased with increasing cellular CAR content in all three species (Fig. 4B, Tables S2 and S3). Notably, *Pseudanabaena* had a similar slope for the relationship between cellular CAR content and TPP/Chl *a* to that of *Microcystis* 912, but the slope was lower than that of *Microcystis* 1028 (Tables S2 and S3; ANOVA, $F_{2,42} = 3.3$, $P < 0.05$).

TABLE 2. Effects of light and iron availability on photosynthetic pigment content and pigment concentrations of *Microcystis* 912, *Microcystis* 1028, and *Pseudanabaena* 1282.

Species	Conditions	Chl *a*, fg · cell^{-1}	CAR, fg · cell^{-1}	PC, fg · cell^{-1}	APC, fg · cell^{-1}	PE, fg · cell^{-1}	TPP, fg · cell^{-1}	Chl *a*, fg · μm^{-3}	CAR, fg · μm^{-3}	TPP, fg · μm^{-3}
Microcystis 912	LL + Fe	48.9 ± 5.2^{a}	26.0 ± 2.9^{a}	237.1 ± 31.7^{a}	200.3 ± 22.5^{a}	—	437.4 ± 45.1^{a}	4.3 ± 0.5^{a}	2.3 ± 0.3^{a}	38.8 ± 4.2^{a}
	LL − Fe	16.6 ± 2.1^{b}	15.5 ± 1.6^{b}	140.2 ± 30.8^{b}	209.1 ± 41.3^{a}	—	349.2 ± 15.1^{b}	1.0 ± 0.1^{b}	1.0 ± 0.1^{b}	21.8 ± 1.2^{b}
	HL + Fe	27.6 ± 0.8^{c}	33.3 ± 2.3^{c}	164.0 ± 15.3^{b}	167.4 ± 21.4^{ab}	—	331.4 ± 36.5^{b}	0.7 ± 0.0^{b}	0.8 ± 0.1^{b}	8.5 ± 1.1^{c}
	HL − Fe	30.1 ± 1.6^{c}	32.6 ± 2.6^{c}	164.6 ± 6.2^{b}	121.3 ± 22.3^{b}	—	286.0 ± 18.2^{b}	0.7 ± 0.0^{b}	0.8 ± 0.1^{b}	7.1 ± 0.8^{c}
Microcystis 1028	LL + Fe	51.0 ± 1.4^{a}	33.0 ± 1.2^{a}	256.0 ± 45.1^{a}	384.4 ± 56.6^{a}	—	640.4 ± 19.8^{a}	1.8 ± 0.0^{a}	1.2 ± 0.0^{a}	22.6 ± 0.7^{a}
	LL − Fe	13.6 ± 2.4^{b}	16.8 ± 1.4^{b}	68.5 ± 2.7^{b}	92.8 ± 15.7^{b}	—	161.3 ± 13.6^{b}	0.6 ± 0.1^{b}	0.8 ± 0.1^{b}	7.7 ± 0.6^{b}
	HL + Fe	50.3 ± 3.9^{a}	49.1 ± 3.7^{c}	148.2 ± 5.4^{c}	228.7 ± 3.9^{c}	—	376.9 ± 9.3^{c}	1.4 ± 0.1^{c}	1.4 ± 0.1^{c}	10.4 ± 0.3^{c}
	HL − Fe	20.7 ± 3.5^{c}	31.7 ± 1.8^{a}	130.7 ± 14.4^{c}	164.4 ± 26.5^{c}	—	295.1 ± 13.7^{d}	0.7 ± 0.1^{b}	1.0 ± 0.1^{d}	9.3 ± 0.4^{c}
Pseudanabaena 1282	LL + Fe	74.1 ± 4.5^{a}	27.2 ± 0.9^{a}	91.4 ± 11.5^{a}	84.9 ± 10.3^{a}	130.7 ± 4.2^{a}	308.2 ± 3.1^{a}	18.4 ± 1.1^{a}	6.8 ± 0.2^{a}	76.4 ± 0.8^{a}
	LL − Fe	22.3 ± 6.8^{b}	18.9 ± 2.0^{b}	64.0 ± 1.9^{b}	61.3 ± 2.2^{b}	78.9 ± 0.5^{b}	204.1 ± 4.6^{b}	4.4 ± 1.3^{b}	3.7 ± 0.4^{b}	40.5 ± 0.9^{b}
	HL + Fe	50.3 ± 5.7^{c}	27.6 ± 1.0^{a}	35.8 ± 5.3^{c}	38.5 ± 3.1^{c}	28.4 ± 2.0^{c}	102.7 ± 4.7^{c}	7.4 ± 0.8^{c}	4.0 ± 0.2^{b}	15.1 ± 0.7^{c}
	HL − Fe	38.7 ± 4.3^{d}	24.1 ± 1.6^{c}	15.8 ± 2.4^{d}	26.6 ± 5.6^{c}	25.0 ± 2.4^{c}	67.3 ± 5.1^{d}	5.8 ± 0.6^{bc}	3.6 ± 0.2^{b}	10.0 ± 0.8^{d}

LL + Fe (low-light and iron-replete conditions), LL − Fe (low-light and iron-limited conditions), HL + Fe (high-light and iron-replete conditions), HL − Fe (high-light and iron-limited conditions). [a, b, c, d] Values with different superscript letters for each species in the same column are significantly different (Tukey's HSD, $P < 0.05$).

Data are mean ± SD (n = 4).

Chl *a*, chlorophyll *a*; CAR, carotenoids; PC, phycocyanin; APC, allophycocyanin; PE, phycoerythrin; TPP, total phycobilin pigments.

DISCUSSION

Our analysis clearly showed that high-light relative to low-light conditions alleviated the negative effect of iron limitation on the growth of *Pseudanabaena*, while it similarly improved the growth of the two *Microcystis* strains under both iron-limited and iron-replete conditions (Fig. 1). These results suggest that the negative effects of iron limitation on growth were highly dependent on light availability in *Pseudanabaena* but not in the two *Microcystis* strains.

Cyanobacteria have evolved efficient light-harvesting strategies by remodeling the size and ratio of their light-harvesting antenna system, the phycobilisomes, in low-light environments (Kehoe and Gutu 2006). Unlike *Microcystis*, *Pseudanabaena* has an additional phycobilin, PE, which can absorb light in the 540–570 nm range of the visible spectrum, conferring a competitive advantage to *Pseudanabaena* in fluctuating light environments (Stomp et al. 2008). Although *Pseudanabaena* has a similar cellular Chl *a* and CAR content and a much lower cellular TPP content when compared with the *Microcystis* strains, their smaller cell size results in a much higher concentration of these photosynthetic pigments in *Pseudanabaena*. These size-dependent patterns of pigment concentration were also observed in other phytoplankton groups (Finkel 2001, Key et al. 2010). Moreover, low-light conditions were associated with a relative increase in antenna-associated pigments (cellular TPP content and TPP concentration) in *Pseudanabaena* when compared with the two *Microcystis* strains. The increased concentration of photosynthetic pigments was associated with the highest photosynthetic efficiency (α) when iron was sufficient (Fig. 2B), indicating that *Pseudanabaena* might have a physiological advantage under low-light conditions if iron is sufficient. A higher $rETR_{max}$ typically implies that more photosynthetic units are required to sustain faster growth rates (MacIntyre et al. 2002, Bernstein et al. 2014), which could also apply to *Pseudanabaena* under low-light conditions in comparison with the two *Microcystis* strains, but only when iron is sufficient. Consequently, the smaller cell size, higher cellular photosynthetic pigment concentration, and higher photosynthetic efficiency of *Pseudanabaena* relative to *Microcystis* boost the light-harvesting capabilities and facilitate the success of *Pseudanabaena* under lower light conditions when cells have enough iron to invest in the biosynthesis of photosynthetic pigments and photosynthetic units.

The higher surface-to-volume ratios of smaller cells facilitate higher cellular iron uptake capacity (Sunda and Huntsman 1997, Raven 1999). However, under low-light conditions, iron limitation caused cell size to increase in both *Pseudanabaena* and *Microcystis* 912, which could have impaired iron uptake and thus stimulated a more serious iron limitation effect on growth. Nevertheless, the cell size of *Microcystis* 1028

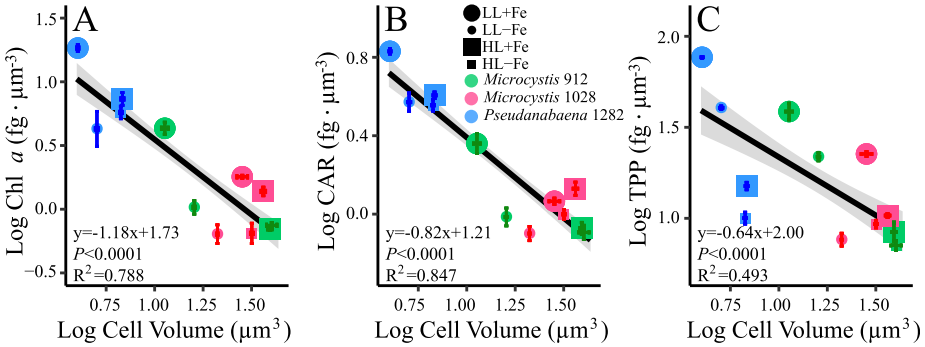

FIG. 3. $\log_{10}$ Chl *a* concentration (A), $\log_{10}$ CAR concentration (B), and $\log_{10}$ TPP concentration (C) of *Microcystis* 912, *Microcystis* 1028, and *Pseudanabaena* 1282 grown under LL + Fe (big-circles symbol, low-light, and iron-replete), LL − Fe (small-circles symbol, low-light, and iron-limited), HL + Fe (big square symbol, high-light, and iron-replete) and HL − Fe (small square symbol, high-light, and iron-limited) conditions as a function of $\log_{10}$ Cell Volume. Chl *a*, chlorophyll *a*; CAR, carotenoids; TPP, total phycobilin pigments. Green, red, and blue colors represent *Microcystis* 912, *Microcystis* 1028, and *Pseudanabaena* 1282, respectively. Linear regressions with $P < 0.05$ are plotted in figures. Shadings represent 95% confidence limits for the regressions. Averages (±SD; n = 4) for each species and each condition are plotted.

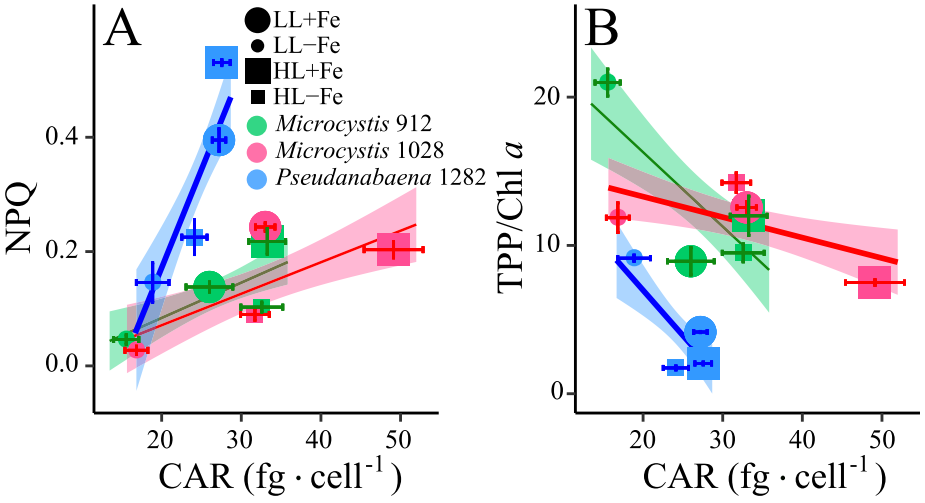

FIG. 4. Nonphotochemical quenching (NPQ) (A) or total phycobilin pigments/chlorophyll *a* ratio (TPP/Chl *a*) (B) as a function of carotenoids (CAR) content of *Microcystis* 912, *Microcystis* 1028, and *Pseudanabaena* 1282 grown under LL + Fe (big-circles symbol, low-light, and iron-replete), LL − Fe (small-circles symbol, low-light, and iron-limited), HL + Fe (big square symbol, high-light, and iron-replete), and HL − Fe (small square symbol, high-light, and iron-limited) conditions. Green, red, and blue colors represent *Microcystis* 912, *Microcystis* 1028, and *Pseudanabaena* 1282, respectively. Linear regressions with $P < 0.05$ are plotted in the figures. Shadings represent 95% confidence limits for the regressions. Averages (±SD; n = 4) for each species and each condition are plotted. Least squares linear regression results are provided in Table S2 and Tukey's HSD comparing slopes between species in Table S3.

decreased under iron limitation, regardless of light availability, suggesting that this strain could be well adapted to iron limitation via changes in cell size. Furthermore, under low-light conditions, iron limitation induced a similar decrease in both cellular Chl *a* and CAR content in all three species, but the decrease in cellular phycobilin pigment content was lower than that for cellular Chl *a* and CAR content in both *Pseudanabaena* and *Microcystis* 912 relative to *Microcystis* 1028. As a result, the highest TPP/Chl *a* occurred under the LL − Fe conditions for *Pseudanabaena* and *Microcystis* 912 among the four light and iron combinations. This elevated TPP/Chl *a* likely increased cellular iron demand and could account for the amplified negative effect of the LL − Fe versus LL + Fe on growth. Additionally, for all three species the lowest $rETR_{max}$ occurred under the LL − Fe treatment, suggesting that iron supply increased light harvesting but not the electron transfer in *Pseudanabaena* and *Microcystis* 912 and this physiological response was ultimately responsible for the light/iron co-limitation observed here. Previous work has shown that the polysaccharides of colonial *Microcystis* can store large amounts of iron, which helps them to physiologically respond to iron limitation (Li et al. 2016). Therefore, although the unicellular form of *Microcystis* 912 was the least sensitive to the LL − Fe treatment, the colonial form of *Microcystis* 912 that

exudes polysaccharides, could have an ecophysiological advantage during blooms when iron is limited and light becomes limiting. In contrast, as *Pseudanabaena* has no colonial form, the enzymes for the synthesis of large amounts of phycobilin pigments appear to be a sink for iron, which would tend to induce more serious iron limitation on *Pseudanabaena* than on *Microcystis* under low-light conditions.

Under HL − Fe conditions, *Pseudanabaena* grew faster than *Microcystis* did under HL + Fe conditions (Fig. 1), indicating that there was no effect of iron limitation on *Pseudanabaena* cell division under high light. We further found that the high-light conditions resulted in a more pronounced decrease in the TPP content of *Pseudanabaena* than that of *Microcystis* (Table 2). These results show that *Pseudanabaena* reduced the biosynthesis of phycobilin pigment more readily under sufficient light intensity, reducing the iron quota and the effect of iron limitation, and supporting its faster growth. Decreasing light harvesting to prevent excess excitation and enhancing photoprotection are common strategies of photosynthetic organisms to adapt to high irradiance. We observed that our high-light conditions induced a decline in the concentration of all cellular photosynthetic pigments for all three species (Table 2). This could indicate a general decrease in all components of the photosynthetic apparatus, including a reduction in the number of thylakoid membranes (Meneghesso et al. 2016, Li et al. 2018). Furthermore, the high-light treatment resulted in an enhanced decrease in phycobilin pigment content and TPP/Chl *a* of *Pseudanabaena* when compared with the two *Microcystis* strains irrespective of the iron conditions. The low TPP and TPP/Chl *a* in *Pseudanabaena* suggests a low amount of light energy is captured and transferred to the reaction centers, resulting in a high saturation irradiance (I_k) and low susceptibility to photoinhibition (also reported by Vonshak et al. 2000).

Carotenoids serve several functions including light harvesting, scavenging of singlet oxygen, stabilization of structures, dissipation of excess energy, and photoprotection (Frank and Cogdell 1996). Various ketocarotenoids are involved in blue-light-induced NPQ to dissipate the excess energy absorbed by the phycobilisomes in cyanobacteria (Wilson et al. 2006, Kerfeld et al. 2017). The significant linear relationship between cellular CAR content and NPQ in all three species (Fig. 4A) indicated that carotenoids play a key role in NPQ in these cyanobacteria. The higher slope between CAR content and NPQ in *Pseudanabaena* than in the two *Microcystis* strains suggested an enrichment of NPQ-related CAR in *Pseudanabaena* (Fig. 3B). *Pseudanabaena* had > 2-fold higher NPQ relative to the two *Microcystis* strains under all treatments, suggesting that *Pseudanabaena* has a higher quenching ability for excess light than *Microcystis*. Moreover, the relationships we observed between NPQ and/or CAR content and growth rate in all three species (Fig. 2, D and E) suggested that higher photoprotection capacity was needed to ensure a higher growth rate when light is sufficient, especially for *Pseudanabaena*, which has larger NPQ and higher growth rate. Therefore, the higher photoprotection capacity of *Pseudanabaena* vs *Microcystis* should be one of the main reasons for its success under high-light conditions.

Overall, when compared with *Microcystis*, distinct strategies were utilized by *Pseudanabaena* to cope with changes in light and iron availability. On the one hand, the excellent light-harvesting ability would provide *Pseudanabaena* with growth advantages under low-light conditions when iron is sufficient, but this species would likely be more susceptible to serious iron limitation in iron-limited water columns. On the other hand, the limited investment in biosynthesis of antenna pigments and photosynthetic components, and the excellent photoadaptation and photoprotection ability under high-light conditions, along with its possible fast metabolism and nutrient uptake because of its small size, probably ensures the ecological success of *Pseudanabaena* in iron-limited conditions.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1. Results of two-way ANOVAs of the effects of light, iron, and their interaction on μ, cell volume, $rETR_{max}$, α, I_k, NPQ, Chl *a*, CAR, TPP, TPP/Chl *a*, Chl *a* concentration, CAR concentration, TPP concentration.

Table S2. Least squares linear regression results for the growth rate (μ) as a function of cell volume, photosynthetic parameters, CAR, and the TPP/Chl *a*, the NPQ, and the TPP/Chl *a* as a function of CAR, and the $\log_{10}$ pigments concentration as a function of $\log_{10}$ cell volume.

Table S3. Results of Tukey's HSD comparing slopes between species reported in Table S2.