Table S1. Results of statistical analysis on the effects of temperature under the same irradiance condition and effects of irradiance at single temperature on several physiological traits by the Tukey's test. The *p* value shows the significance of the multiple comparison. The alphabet represents the significance between different treatments, with the same alphabet in one multiple comparisons indicating no significant difference between different treatments.

Parameter	Temperature effect							Irradiance effect						
	I	*p* value	19	22	25	28	31	T	*p* value	20	80	140	220	320
ETR	20	<0.0001	a	a	b ↓	c ↓	d ↓	19	<0.0001	a	b ↑	c ↑	d ↑	d
	80	<0.0001	a	b ↓	c ↓	d ↓	e ↓	22	<0.0001	a	b ↑	c ↑	d ↑	e ↑
	140	<0.0001	a	b ↑	a ↓	a	c ↓	25	<0.0001	a	b ↑	c ↑	d ↑	e ↑
	220	<0.0001	a	b ↑	c ↓	c	d ↓	28	<0.0001	a	b ↑	c ↑	d ↑	d
	320	<0.0001	a	b ↑	c ↓	d ↓	e ↓	31	<0.0001	a	b ↑	c ↑	d ↑	e ↓
Ingestion rates	20	<0.0001	a	b ↑	c ↑	d ↑	e ↑	19	<0.0001	a	a	ab	b	c ↓
	80	<0.0001	a	b ↑	c ↑	d ↑	e ↓	22	<0.0001	a	b ↑	c ↑	d ↓	e ↓
	140	<0.0001	a	b ↑	c ↑	d ↑	c ↓	25	<0.0001	a	b ↑	c ↑	d ↓	e ↓
	220	<0.0001	a	b ↑	c ↑	d ↑	e ↑	28	<0.0001	a	b ↑	c ↑	d ↓	d
	320	<0.0001	a	b ↑	c ↑	d ↑	e ↑	31	<0.0001	a	b ↓	c ↓	d ↓	e ↓
Growth rates	20	<0.0001	a	b ↑	cd ↑	c	d ↓	19	<0.0001	a	b ↑	c ↑	d ↑	e ↑
	80	<0.0001	a	b ↑	c ↑	d ↑	c ↓	22	<0.0001	a	b ↑	c ↑	c	d ↑
	140	<0.0001	a	b ↑	c ↑	d ↑	b ↓	25	<0.0001	a	b ↑	b	c ↑	b ↓
	220	<0.0001	a	b ↑	c ↑	a ↓	d ↓	28	<0.0001	a	b ↑	b	c ↓	d ↓
	320	<0.0001	ab	a	b ↓	c ↓	d ↓	31	<0.0001	a	b ↑	b	c ↓	d ↓
Cellular Chl *a* concents	20	<0.0016	a	b ↓	b	b	b	19	<0.0001	a	b ↓	c ↓	d ↓	e ↓
	80	<0.0001	a	b ↓	b	b	c ↓	22	<0.0001	a	b ↓	b	b	c ↓
	140	<0.0001	a	b ↓	c ↓	c	d ↓	25	<0.0001	a	b ↓	c ↓	d ↓	d
	220	<0.0001	a	b ↓	c ↓	c	c	28	<0.0001	a	b ↓	c ↓	c	c
	320	<0.0001	a	a	b ↓	b	b	31	<0.0001	a	b ↓	b	b	b

Inorganic carbon uptake rates	20	0.006	a	a	b ↑	a ↓	ab	19	<0.0001	a	-	b ↑	-	c ↑
	80	-	-	-	-	-	-	22	<0.0001	a	-	b ↑	-	-
	140	<0.0001	a	a	b ↓	c ↓	d ↓	25	<0.0001	a	-	b ↑	-	c ↑
	220	-	-	-	-	-	-	28	<0.0001	a	-	b ↑	-	c ↑
	320	<0.0001	a	-	b ↑	c ↓	d ↓	31	0.0001	a	-	b ↑	-	c ↑
Chl *a* specifc-inorgainc carbon uptake rates	20	<0.0001	a	b ↑	c ↑	bc	c	19	<0.0001	a	-	b ↑	-	c ↑
	80	-	-	-	-	-	-	22	-	a	-	b ↑	-	-
	140	<0.0001	a	b ↑	c ↑	c	b ↓	25	<0.0001	a	-	b ↑	-	c ↑
	220	-	-	-	-	-	-	28	-	a	-	b ↑	-	c ↑
	320	<0.0001	a	-	b ↑	a ↓	c ↓	31	<0.0001	a	-	b ↑	-	c ↑

*Arrows behind the alphabet indicate the direction of significant response compared with its adjacent previous treatment. For example, when checking the temperature effect on ETR under 20 μmol m^{-2} s^{-1}, the downward arrow behind 'c' suggests ETR at 28 °C is significantly lower than that at 25°C.

*'-' represents no data obtained under this treatment.

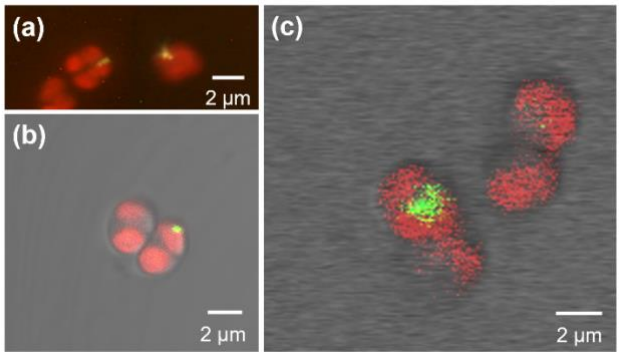

Fig. S1. *Picochlorum* sp. with ingested bacteria. (**a**) FLB phagocytosed by *Picochlorum* sp. observed under epifluorescence microscopy (Olympus BX51). (**b**) *Picochlorum* sp. with ingested FLB observed by confocal microscope. (**c**) *Picochlorum* sp. dyed with lysotracker green shows acidic food vacuole-like compartment in this green alga. Images in **a** and **b** were captured with LSM confocal microscope in our previous study (Pang et al. 2022).

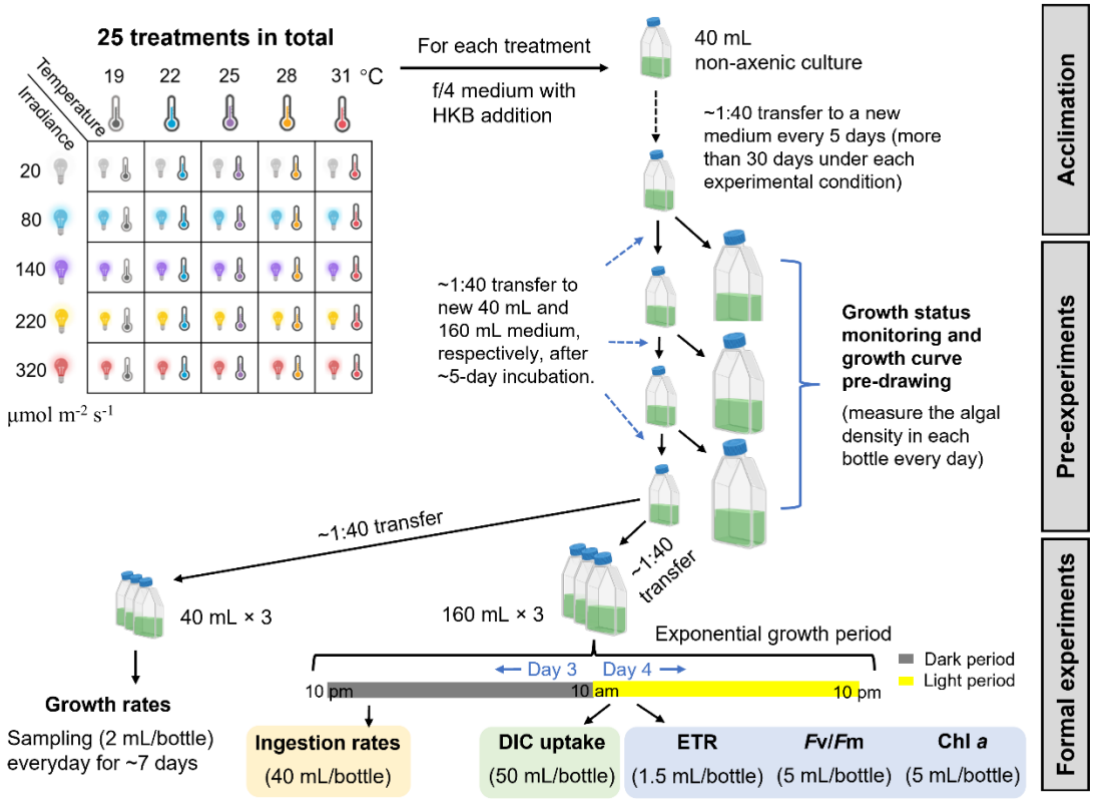

Fig. S2. Schematic diagram of the experimental setting and parameter measurement. There were 25 treatments (5 temperature × 5 irradiance) in our study. In each treatment, we cultured the non-axenic *Picochlorum* sp. in nutrient (f/4 media) and prey (heat-killed bacteria) sufficient condition throughout the experiment. Algal acclimation lasted for more than 30 d. During this period, alga in each treatment was cultured in a 40-mL medium in the well-ventilated culture flask and transferred every 5 d, with the inoculation ratio of algal culture to a new medium around 1:40. After acclimation, we conducted a series of pre-experiments to monitor algal growth, pre-drew growth curve, and compared growth conditions in 40-mL and 160-mL systems. The reason we incubated the culture and tested the cell density in these two size mediums was we expected that samples for all the physiological rates measurement in different treatments could be conducted simultaneously, which need a great space of incubator. Under this scenario, for each treatment, we can only put into three 40-mL flasks for growth testing and three 160-mL culture for other parameter estimation. Therefore, pre-experiments for the growth rates and status of the alga testing in the two bottles was necessary. Specifically, for each treatment, we transferred the well-acclimated *Picochlorum* sp. to new 40-mL and 160-mL medium (bottle) and measured the cell density in each bottle daily using the flow cytometer. The monitoring session lasted around 5 d. After that, we transferred the culture from the 40-mL bottle to another new 40-mL and 160-mL medium and redid the monitoring session again twice. Our pre-experiments showed that the growth of *Picochlorum* sp. in the two systems (i.e., 40 mL and 160 mL bottle) were not significantly different. On the other hand, as the estimation of DIC uptake rates requires 4-h incubation, in our pre-experiments, we also carried out a time-series cell density monitoring during the exponential growth period to identify the

experimental period in later formal experiments (Detailed information can be found in Fig. S4). Subsequently, in the formal experiments, we transferred the well-acclimated algal culture in each treatment to three 40-mL flasks to calculate the specific growth rates (n=3) and to three 160-mL flasks to estimate other physiological parameters (n=3), with the initial algal density in each bottle around 5×10^4 cells mL^{-1}. To calculate the growth rates during the exponential phase, after inoculation, 2 mL algal culture was sampled from each 40-mL culture flask every day and lasted for ~7 d, which enabled us to obtain the relative complete growth curve (i.e., lag phase, exponential phase, then growing slowly and entering the stationary phase) and find the exponential phase (usually started on Day 3 after inoculation and lasted for 2~3 d). Samples for other parameter measurements were cultured in the 160-mL culture flask. After entering the exponential growth phase, we sampled three times from the 160 mL flask. The first sampling was for the ingestion rates estimation (40 mL/bottle), which was conducted 2 h after entering the dark period on Day 3. Then was the collection of the samples for DIC uptakes (50 mL/bottle), which was conducted in the next morning (0.5 h after entering the light period). The last sampling happened 2 h after entering the light period on Day 4, where samples for estimating ETR (1.5 mL), *F*v/*F*m (5 mL), and Chl*a* (5 mL) were collected simultaneously. The entire sampling operations were carried out gently to not interfere with estimating the remaining parameters.

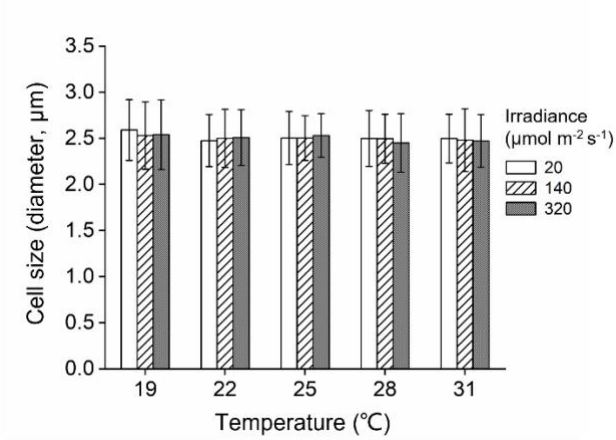

Fig. S3. Cell size of *Picochlorum* sp. in different temperature and irradiance conditions. Cell size under 20, 140, and 320 μmol m^{-2} s^{-1} at all the experimental temperatures was analyzed using the inverted microscope (IX 51 Olympus) and measured with the software SPOT ADVANCED. Forty *Picochlorum* sp. cells in each treatment were randomly selected for size estimation (n = 40), with the error bar representing standard deviation of the mean. The results showed no significant difference in the algal cell size between different treatments (Tukey's test, $p > 0.05$).

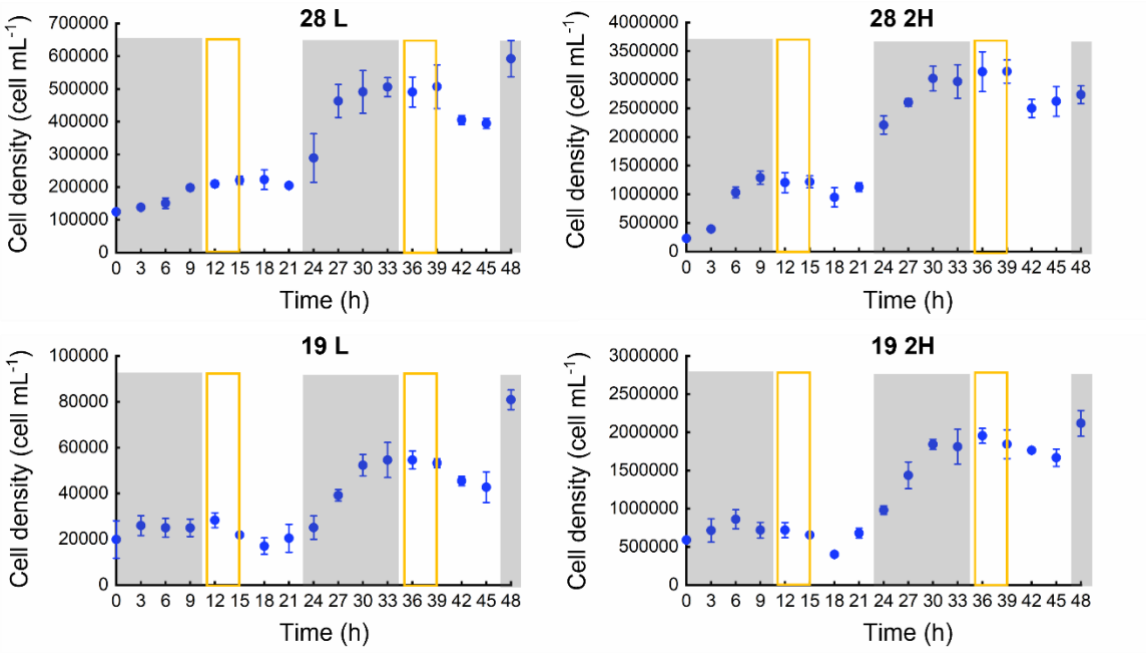

Fig. S4. The variation of daily algal cell density at 19 and 28°C under 20 (L) and 220 µmol m^{-2} s^{-1} (2H) in 48 h monitoring during the exponential growth phase by pre-experiments. The white and grey parts represent the light and dark periods, respectively. The algal density was measured every 3 h with the flow cytometer, with the error bar representing the standard deviation of the mean from two biological replicates. The orange frames represent the cell density variation from 0.5 h after entering the light period to the next 4 h, which shows a relatively small change. As such, we decided to carry out the short-term (4 h) incubation in this period in the later formal experiment to estimate the DIC uptake rates.

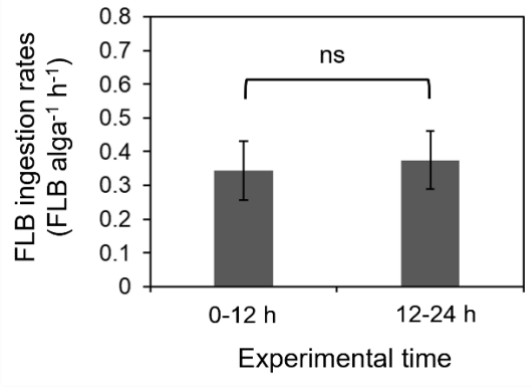

Fig. S5. Average FLB ingestion rates in the first and last 12 h of the day. Data from the previous study about prey reduction experiment on *Picochlorum* sp. (Pang et al. 2022). The first 12 h includes 10-h dark period and 2-h light period, while the last 12 h contained 10-h light period and 2-h dark period. "ns" represents no significant difference between these two periods. The error bar indicates the standard deviation of the mean from twelve biological replicates.

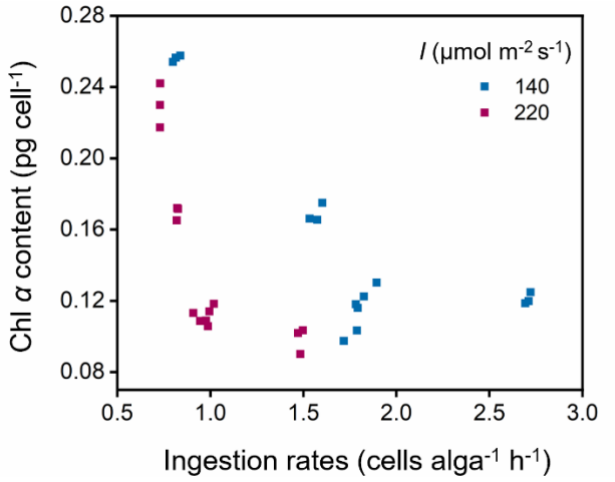

Fig. S6. Relationship of Chl *a* content and ingestion rates under 140 and 220 µmol m^{-2} s^{-1}. The overall negative correlations between these two traits showed a trade-off between photosynthesis and ingestion of *Picochlorum* sp.

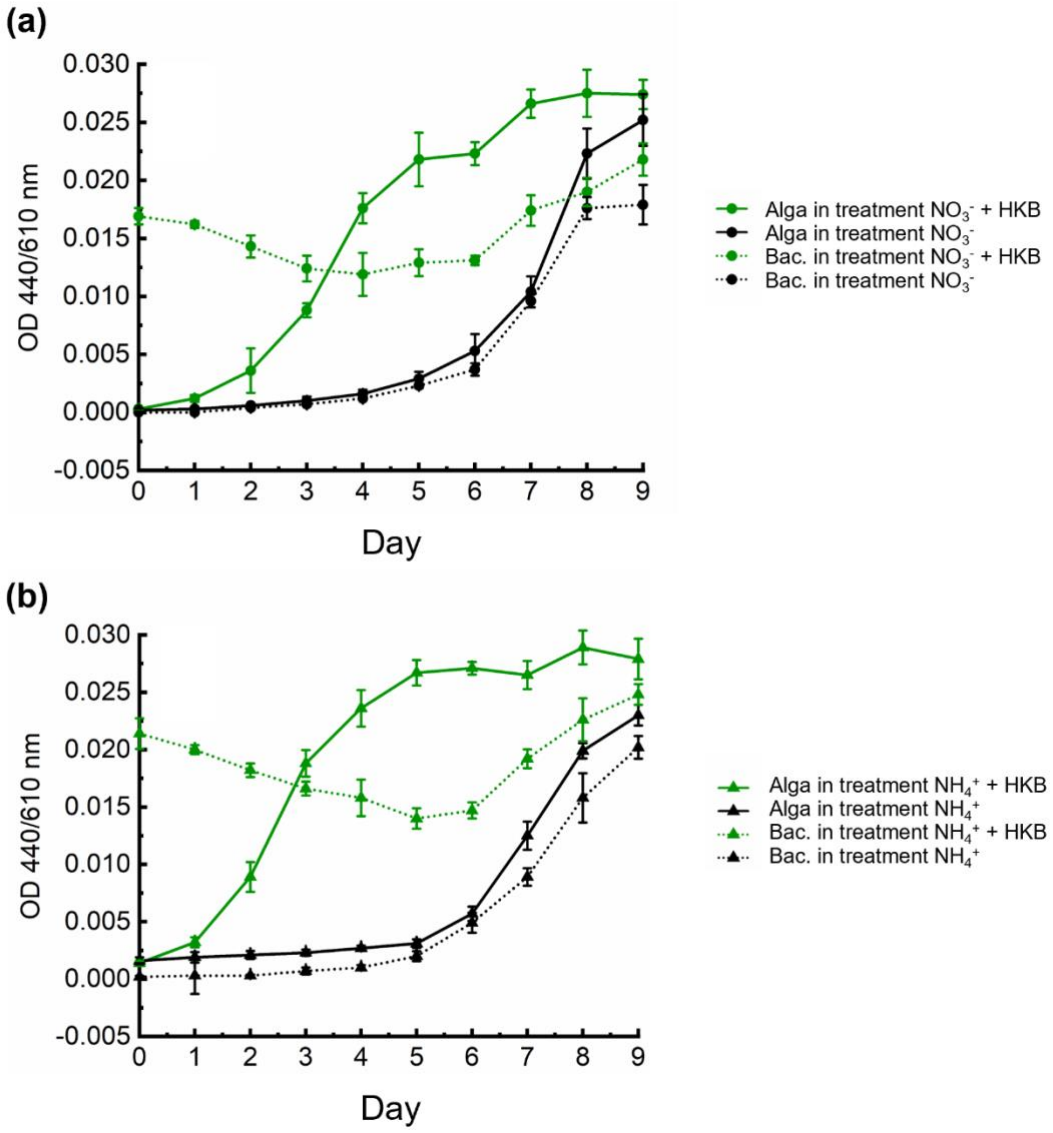

Fig. S7. Growth curves of alga and bacteria in two nitrogen sources. (**a**) *Picochlorum* sp. cultured with the nitrate-based f/40 (20× dilution of f/2 media) medium. (**b**) *Picochlorum* sp. cultured with the ammonium-based f/40 medium in which sodium nitrate ($NaNO_3$) was replaced by ammonium chloride (NH_4Cl). The green lines in **a** and **b** represent treatments with prey (heat-killed bacteria, HKB) addition; the black lines indicate prey-unamended treatments. The relative cell density variation of alga and bacteria is represented by the OD values measured under 440 and 610 nm, respectively. The error bar represents the standard deviation of the mean from three biological repeats. The algal solutions were treated with antibiotics before inoculation. Our previous study identified *Picochlorum* sp. as an obligate mixotroph as it cannot survive in complete darkness and prey-depleted conditions (Pang et al. 2022). During that time, the alga was incubated in f/4 medium (2× dilution of f/2 medium), where nitrate was the only nitrogen source. However, recent work found that the pre-identified obligate mixotroph, Karlodinium armger, actually can grow autotrophically in the ammonium-based medium (Binzer et al. 2020), indicating it was incorrect to use

"obligate mixotroph" to describe this mixotroph. As such, we conducted this experiment to test whether *Picochlorum* sp. strictly follows the "obligate mixotroph" term by culturing them under prey-depleted conditions in the ammonium-substituted nitrogen medium. Specifically, after well-acclimated under the nitrate- and ammonium-based f/40 medium (~44.1 μM DIN; at 25°C under 100 μmol m^{-2} s^{-1}), the non-axenic culture in the exponential growth phase was treated with 10 mg L^{-1} antibiotics (i.e., antibiotic combinations of 5 mg L^{-1} Cephalothin and 5 mg L^{-1} Ampicillin) for 6 h, then followed by several times centrifuging, washing, and resuspending with sterile artificial seawater to remove the antibiotics and most of the bacteria. Specific operations and the antibiotic type and concentration were referred to Pang et al. (2022). After that, we transferred the resuspending algal culture to the corresponding new bacteria-free f/40 medium. We separated each nitrogen source into two groups, one with sufficient prey (i.e., ~1×10^7 HKB mL^{-1}) addition and another without prey addition. The bacteria for HKB making were collected from the non-axenic culture in the corresponding nitrogen source, then heat-killed, concentrated, washed, and resuspended referring to Wilken et al. (2020). Overall, there were four treatments (2 nitrogen sources × 2 prey conditions), with each treatment including three biological replicates. All the culture flasks were shaken several times every day. We sampled daily for 9 d and measured the OD value with Spectrophotometer UV-1900i (SHIMADZU) under 440 and 610 nm, respectively, to get the relative cell density variation of alga (440 nm) and total bacteria (610 nm). Our results showed that *Picochlorum* sp. in prey-unamended treatments barely not grew in the first four to five days in both ammonium-based and nitrate-based sources, whereas it grew gradually when the ambient bacteria started increasing. However, in prey-added treatments, *Picochlorum* sp. grew rapidly once inoculation and came into the stationary phase around Day 5. At the same time, the HKB decreased due to the grazing of *Picochlorum* sp., while the bacterial abundance increased from Day 7 because of the growth of free-living bacteria. Combined, it is indicated that *Picochlorum* sp. cannot grow without prey in both ammonium-based and nitrate-based nitrogen medium. Combined with our previous testing that *Picochlorum* sp. cannot live under total darkness with replete prey, it is indicated that *Picochlorum* sp. is indeed an obligate CM.

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