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Heavy precipitation-induced Yangtze River runoff greatly regulates heterotrophic prokaryotes production and induces P-limited growth in the northern East China Sea

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Materials and methods

S1. FDOM analysis method

For measuring the fluorescence spectra of fluorescent dissolved organic matter (FDOM) samples, a Hitachi F-7100 fluorescence spectrophotometer (Hitachi, Tokyo, Japan) was used. Excitation-emission matrix spectroscopy (EEMS) analysis was performed by measuring excitation wavelengths (Ex) from 250 to 500 nm (5 nm intervals) and emission wavelengths (Em) from 300 to 500 nm (5 nm intervals) with an integration time of 0.1 s. To prevent the inner filter effect, absorbance at 254 nm was checked; it stayed below 0.3 for all samples, thus no dilution was needed (Burdige et al., 2004). Scanning was conducted using a Lambda 365 UV/vis spectrophotometer (Perkin Elmer, Norwalk, USA). Milli-Q water EEMS data were used for blank subtraction and for normalizing fluorescence intensity into Raman units (R.U.) (Lawaetz and Stedmon, 2009). Parallel factor analysis (PARAFAC) was performed on 460 sets of EEMS data using MATLAB R2024a software (MathWorks Inc, Natick, USA) with the DOMFluor toolbox (Stedmon and Bro, 2008). Rayleigh and Raman scatter bands (±20 nm) were removed and replaced with missing values ("NaN" in MATLAB). Validation of the 3-component PARAFAC model was conducted through split-half validation and random initialization (Stedmon and Bro, 2008), both showing a percentage of explained variance of 98.1% and 98.0%, respectively. The results characterized one terrestrial humic-like (Component 1; C1) and two protein-like fluorescent components (Component 2 and 3; C2 and C3) in northern East China Sea (Fig. S6). The characterized three fluorescent components (C1 to C3) were compared with those from previous studies in the OpenFluor database, with Tucker's congruence coefficients exceeding 0.95, matching with the major components from 101, 76, and 32 studies, respectively (Table S3, https://openfluor.lablicate.com, last access: 24 September 2024) (Murphy et al., 2014). Component 1 ($FDOM_H$; Ex/Em = 250/430) is categorized as a mixture of terrestrial humic-like FDOM (peak A; Coble, 1996) and marine humic-like FDOM (peak M; Coble, 1996), and it can commonly be monitored in low salinity areas, such as the Yangtze River estuary in the East China Sea (Jiang et al., 2016; Zheng et al., 2018; Li et al., 2020; Sun et al., 2022; Ji et al., 2024). Component 2 ($FDOM_T$; Ex/Em = 280/340) is categorized as Tryptophan-like FDOM (peak T; Coble, 1996), and Component 3 ($FDOM_B$; Ex/Em = 270/300) is categorized as Tyrosine-like FDOM (peak B; Coble, 1996). The humification index (HIX) was calculated as the ratio of the fluorescence intensity area at emission wavelengths 435–480 nm to 300–345 nm, at excitation wavelength 255 nm (Zsolnay et al., 1999)

S2. Primary production

Phytoplankton primary production (PP) was assessed using stable carbon isotope (^{13}C) analysis, following the methodology outlined by Hama et al. (1983). Water samples were obtained from six distinct photic depths, representing 100%, 50%, 30%, 12%, 5%, and 1% penetration of surface photosynthetically active radiation (PAR), determined through the conversion of Secchi disc depth measurements at each sampling station. During the incubation experiments, water samples from each light depth were promptly transferred to 1 L polycarbonate incubation bottles equipped with optical filters (neutral density screens, Lee Filters; Garneau et al., 2007) to replicate in-situ light conditions corresponding to the depths of sample collection. To mitigate potential grazing impacts from large zooplankton during the incubation period, 333 μm sieves were employed. Subsequently, ^{13}C-labelled sodium bicarbonate ($NaH^{13}CO_3$), comprising approximately 10% of concentrations in the ambient dissolved inorganic carbon, was introduced into the bottles containing the water samples. These inoculated samples were then incubated in a large on-deck incubator, maintaining light and temperature conditions consistent with those present at the sea surface. Incubations were terminated within 4-6 hours, followed by filtration through 25 mm GF/F filters (Whatman, 0.7 μm pore size) that had been pre-treated by combustion at 450 °C for 4 hours. After overnight exposure to HCl fumes to remove carbonate, the samples were analyzed at the Alaska Stable Isotope Laboratory, University of Alaska, Fairbanks, USA. Particulate organic carbon and the abundance of ^{13}C were measured using the Finnigan Delta + XL mass spectrometer. These data were then utilized to calculate primary production at each light depth according to the equation suggested by Hama et al. (1983). Depth-integrated primary production (mg C m^{-2} h^{-1}) was computed by applying trapezoidal integration to integrate volumetric primary production (mg C m^{-3} h^{-1}) throughout the entire photic zone. The daily PP (mg C m^{-2} d^{-1}) was determined by combining the hourly primary production observed in this study with previously reported 10-hour photoperiods per day in adjacent regional seas (Jang et al., 2018, 2021; Lee et al., 2017).

S3. Heterotrophic prokaryotes production

The Heterotrophic prokaryotes production (HPP) was determined by measuring the rate of protein synthesis using ^{3}H-leucine (^{3}H-leu) incorporation (Smith and Azam, 1992). Seawater samples (1.5 mL) were incubated at in situ temperature with ^{3}H-leu (final concentration, 10 nM, Perkin Elmer, NET1166005) for one hour in the dark. After incubation, cold 50% trichloroacetic acid (TCA) was added to stop the incubation, and samples were kept in the dark at room temperature for 30 minutes. Blank samples were treated similarly but with the addition of cold 50% TCA before the injection of ^{3}H-leu. After incubation, samples were centrifuged at 14,000 rpm for 10 minutes, and the supernatant was carefully extracted without disturbing the substances adhering to the tube walls. Cold 5% TCA was added to the tubes to extract the synthesized protein, and after another centrifugation at 14,000 rpm for 10 minutes, the supernatant was discarded. The remaining pellet was washed with cold 80% ethanol, followed by another centrifugation at 14,000 rpm for 10 minutes. Liquid scintillation cocktail (1.5 mL; Ultima Gold, Ultima Gold LSC Cocktail) was added to the samples, and ^{3}H radioactivity within the extracted protein was measured using a liquid scintillation counter (LKB, Rack Beta II). The working stock's radioactivity should be 4,995,000 dpm (disintegration per minute). We checked that all working stock radioactivity was within 2% of this value before use. The calculated protein synthesis rate of ^{3}H-leu (pmol leu L^{-1} h^{-1}) was converted to HPP (µg C L^{-1} d^{-1}) using a conversion factor (CF = 1.5 kg C mol leucine^{-1}; Kirchman, 1993).

S4. Statistics

Normality tests were performed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. An independent samples t-test was carried out to compare averaged DOC values within the mixed layer depth (MLD), averaged PP, PA and HPP values within the euphotic depth, and integrated HPP within the MLD in the spring and summer across the ECS, YS, and ES. One-way analysis of variance (ANOVA) was used to compare the means of August DOC and HPP across years, followed by Dunn's post hoc test. In cases where normality assumptions were not met, the Kruskal-Wallis H test was employed. Simple regression analysis was conducted between DOC and salinity within the MLD after confirming the normality and homoscedasticity of residuals. Correlation analyses between PA and DOC, PA and Chl-*a*, HPP and temperature, HPP and Chl-*a*, HPP and DOC, $FDOM_H$ and DOC, $FDOM_H$ and salinity were conducted using Pearson's or Spearman's rank correlation, depending on the normality of the data. A *p*-value of less than 0.05 was considered significant.

Table S1. Seasonal temperature and salinity ranges used to define major water masses in the northern East China Sea in this study (Gong et al., 1996; Hur et al., 1999; Kim and Youn, 2023). KSW: Kuroshio source water, SMW: Shelf mixed water, YRDW: Yangtze River diluted water, TWW: Tsushima warm water, TWC: Taiwan warm current.

	Seasonal temperature (℃) and salinity (psu) range				
	KSW	SMW	YRDW	TWW	TWC
Winter	8.4 < T < 24.5 33.6 < S < 35.2	8.8 < T < 15 32.4 < S < 33.6			
Spring	11.5 < T < 29.4 33.5 < S < 35.2	12 < T < 21 31.2 < S < 33.5			
Summer		14 < T < 23 31 < S < 34	23 < T S < 31	14 < T 34 < S	23 < T 31 < S < 34.2
Autumn	11.8 < T < 27.3 33.4 < S < 34.9	14.3 < T < 24.2 31.9 < S < 33.4			

Table. S2. Spearman's rank correlation coefficients (ρ) between heterotrophic prokaryotes parameters (production, HPP; abundance, PA) and temperature, chlorophyll *a* (Chl-*a*), and dissolved organic carbon (DOC) pooled over three years in the northern East China Sea.

Parameter	HPP	PA
Temperature	ρ = 0.525 ($p < 0.001$)	ρ = 0.364 ($p < 0.001$)
Chl-*a*	ρ = 0.398 ($p < 0.001$)	ρ = 0.281 ($p < 0.001$)
DOC	ρ = 0.457 ($p < 0.001$)	ρ = 0.255 ($p < 0.001$)

Table S3. Characteristics of three PARAFAC components

Component	Ex/Em (nm)	Coble (1996) Peaks	Similar component	Description
C1	250/430	A + M (mixture of terrestrial and marine humic-like)	C1 (Jiang et al., 2016) C4 in summer (Zheng et al., 2018) C2 (Li et al., 2020) C3 (Sun et al., 2022) C3 (Ji et al., 2024)	UVC humic-like components, characterized by high molecular weight, are related to the activity of organisms and are typically found in forest environments and wetlands
C2	280/340	T (Tryptophan-like)	C2 (Jørgensen et al., 2011) C5 (Yamashita et al., 2011) C4 (Cawley et al., 2012) C5 (Asmala et al., 2018)	UVB protein-like components, characterized by high molecular mass DOM, are predominantly derived from autochthonous processes and usually observed in surface waters
C3	270/300	B (Tyrosine-like)	C1 (Murphy et al., 2006) C6 (Yamashita et al., 2011) C4 (Kowalczuk et al., 2013) C1 (Paerl et al., 2020)	Relatively lower molecular mass than tryptophan-like DOM, strongly correlates with total hydrolysable amino acids (THAA)

Table S4. List of acronyms and abbreviations used in this study

Acronym	Full term
DIN	dissolved inorganic nitrogen
DIP	dissolved inorganic phosphorus
DOC	dissolved organic carbon
DOM	dissolved organic matter
ECS	East China Sea
FDOM	fluorescent dissolved organic matter
$FDOM_H$	humic-like fluorescent dissolved organic matter
HIX	humification index
HP	heterotrophic prokaryotes
HPP	heterotrophic prokaryotes production
MLD	mixed layer depth
nECS	northern East China Sea
PA	heterotrophic prokaryotes abundance
PP	primary production
TWC	Taiwan warm current
TWW	Tsushima warm water
YRDW	Yangtze River diluted water

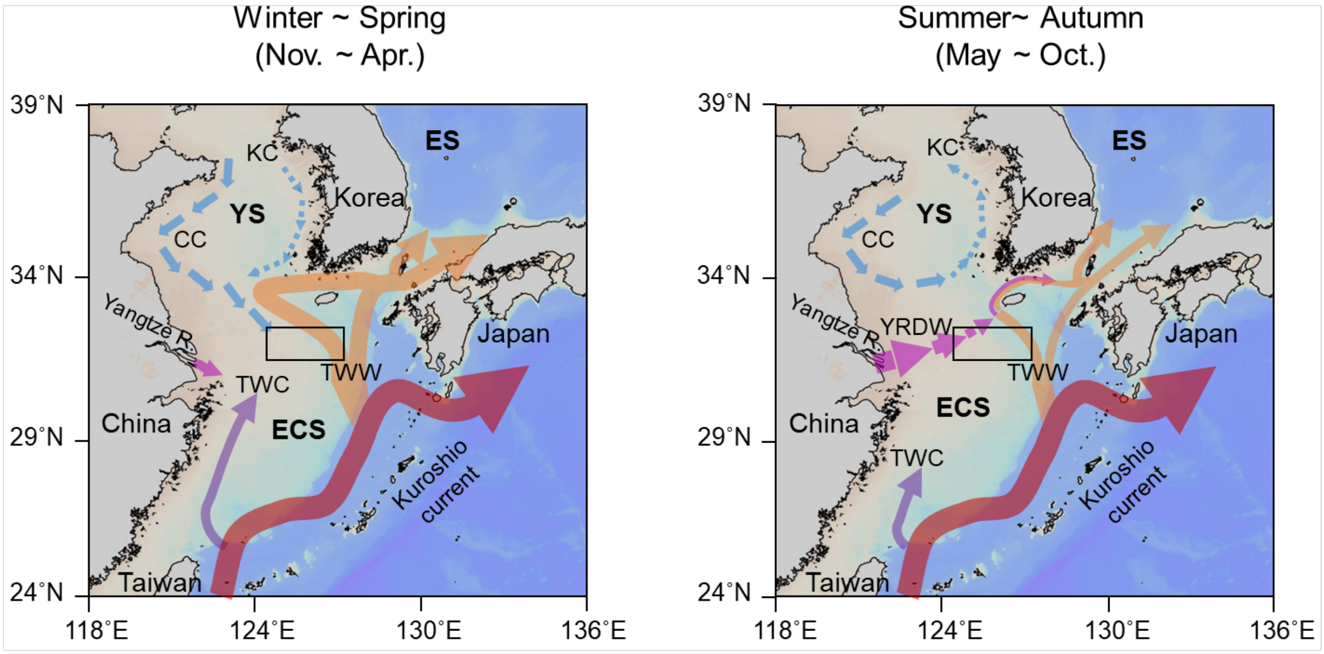


Figure S1. Seasonal surface currents of the Northwest Pacific Ocean. YS: Yellow Sea, ECS: East China Sea, ES: East Sea, CC: Chinese coastal current, KC: Korean coastal current, YRDW: Yangtze River diluted water, TWC: Taiwan warm current, TWW: Tsushima warm water. The winter season spans from November to April, and the summer season from May to October. The black box indicates the study area in the northern East China Sea. Figures were modified from Lie and Cho (2016). Thickness of the arrows denotes magnitude of the current. The Kuroshio current delivers warm and saline water along the shelf break throughout the year. Two major branches of this current, the TWC and the TWW, transport South China Sea water into the central and nECS and subsequently toward the East Sea (Su and Weng, 1994). In winter, the CC, driven by the winter monsoon (i.e., northeasterly winds), brings cold and fresh water southward along the Chinese coast (Chu et al., 2005; Lie and Cho, 2016). In contrast, during summer, the summer monsoon (i.e., southeasterly winds) and river discharge drive the offshore expansion and long-distance transport of the YRDW toward the East Sea (Chang and Isobe, 2003).

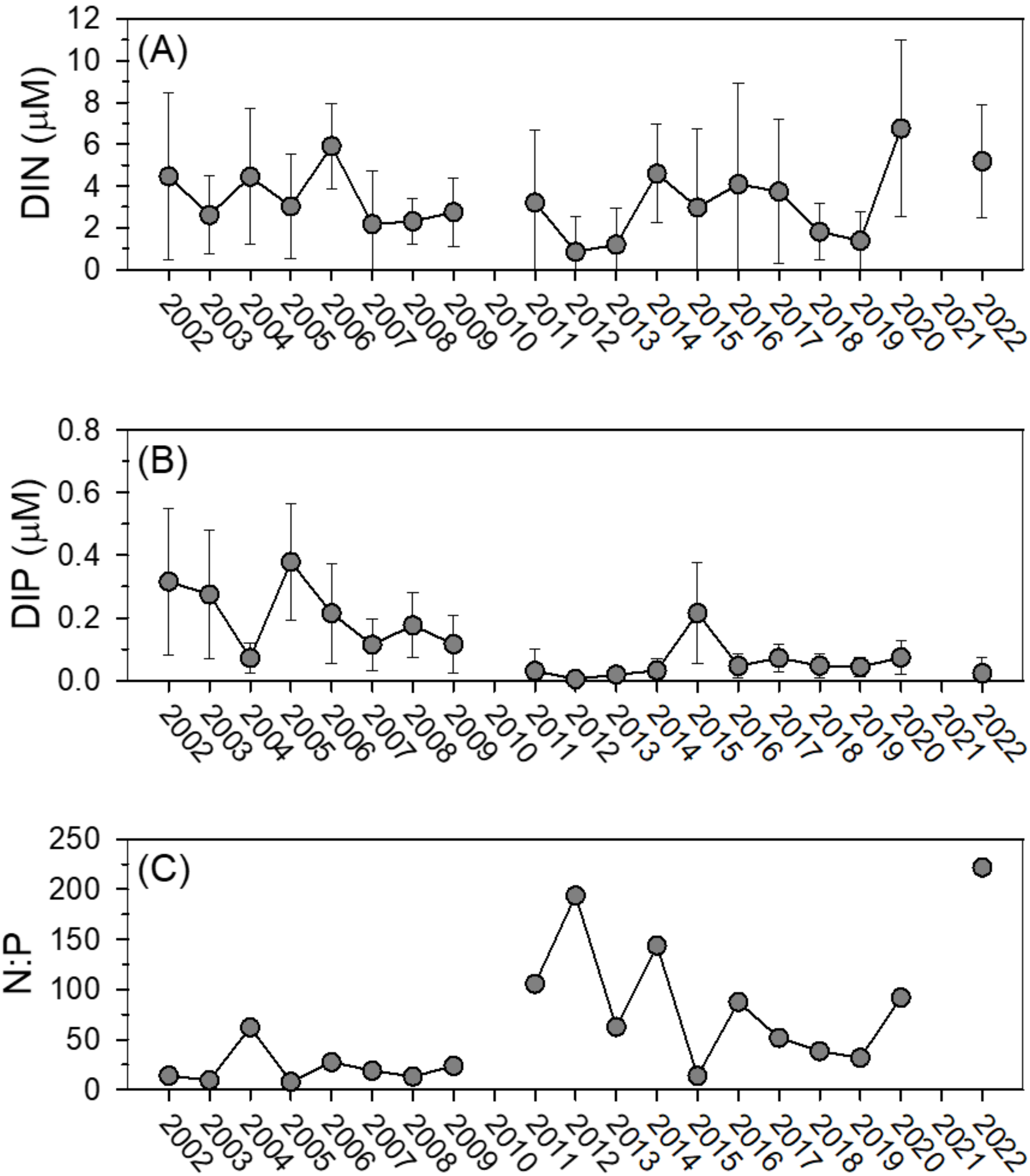


Figure S2. Long-term summer (August) surface (0 – 10 m) nutrient data in the northern East China Sea. (A) Dissolved inorganic nitrogen (DIN, NO_2^-+NO_3^-), (B) dissolved inorganic phosphate (DIP), and (C) DIN to DIP ratio (N:P). Nutrient data were obtained from the Korean National Institute of Fisheries Science (https://www.nifs.go.kr/kodc/soo_list.kodc, last access: 05 November 2024).

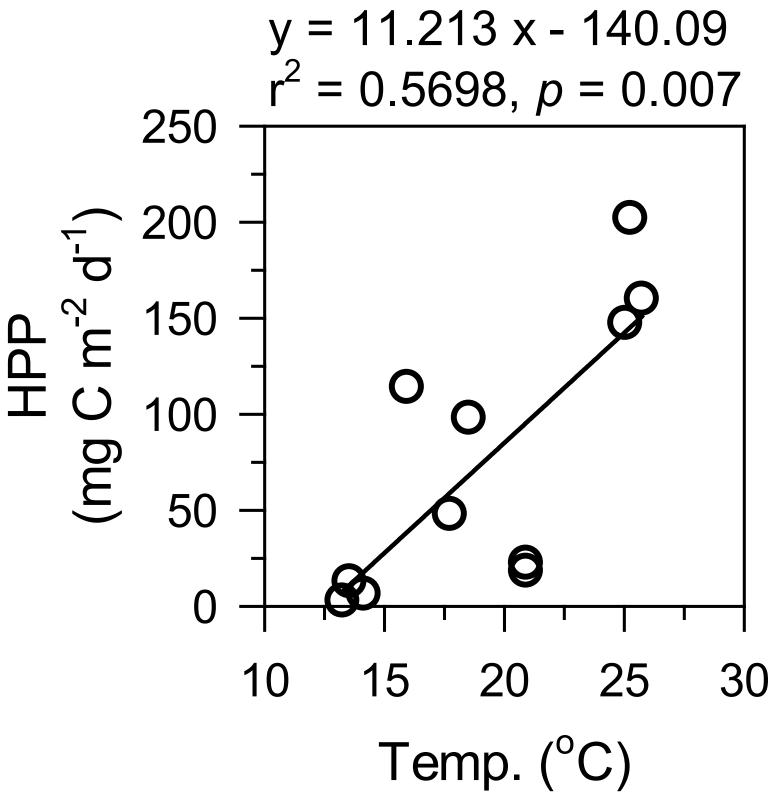
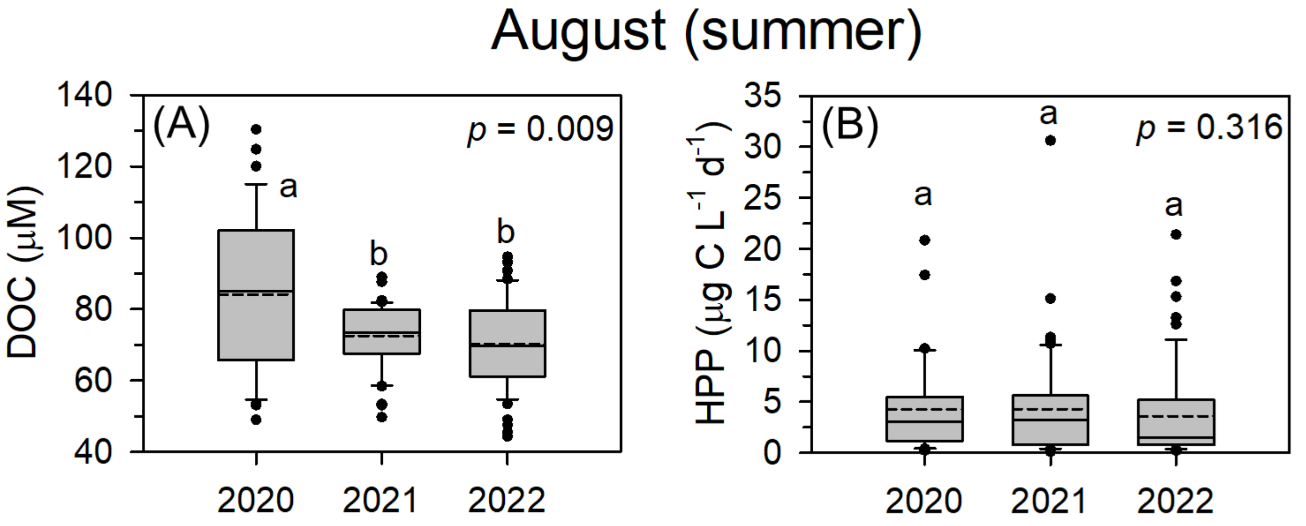


Figure S3. Relationship between the average temperature within the euphotic zone and heterotrophic prokaryotes production (HPP) integrated over the euphotic zone in the northern East China Sea, using seasonally averaged values from 2020 to 2022.



145 Figure S4. Box plot of dissolved organic carbon (DOC) (A) and heterotrophic prokaryotes production (HPP) (B) in August 2020 – 2022. The solid line in each box indicates the median value, and the dotted line indicates the average value (sample size, n = 36 – 55).

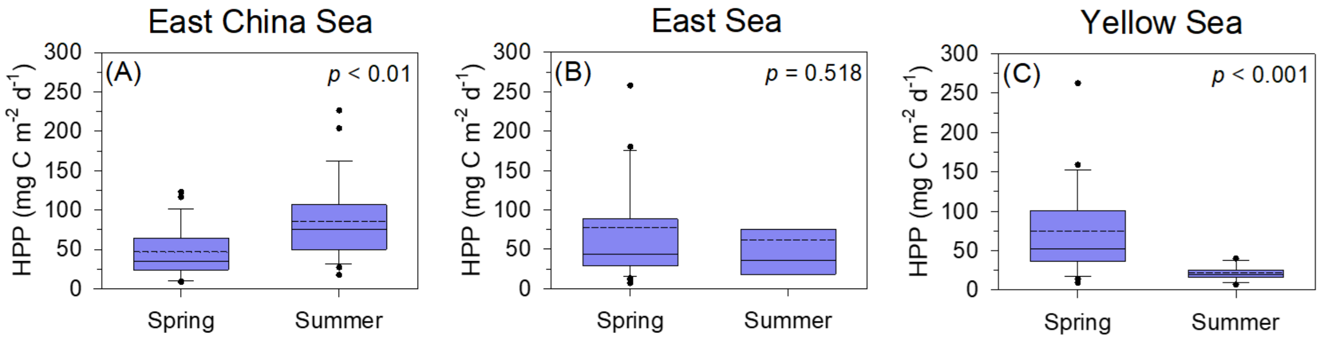


Figure S5. Comparison of integrated heterotrophic prokaryotes production (HPP) within the mixed layer depth in the East China Sea (A), the East Sea (B) and the Yellow Sea (C) during spring and summer from 1996 to 2022 (Hyun and Kim, 2003, Hyun et al., 2009; Kim et al., 2017, 2020, 2025; Hyun J-H unpublished data). The solid line indicates the median value, and the dotted line indicates the average value (sample size, n = 8 – 32).

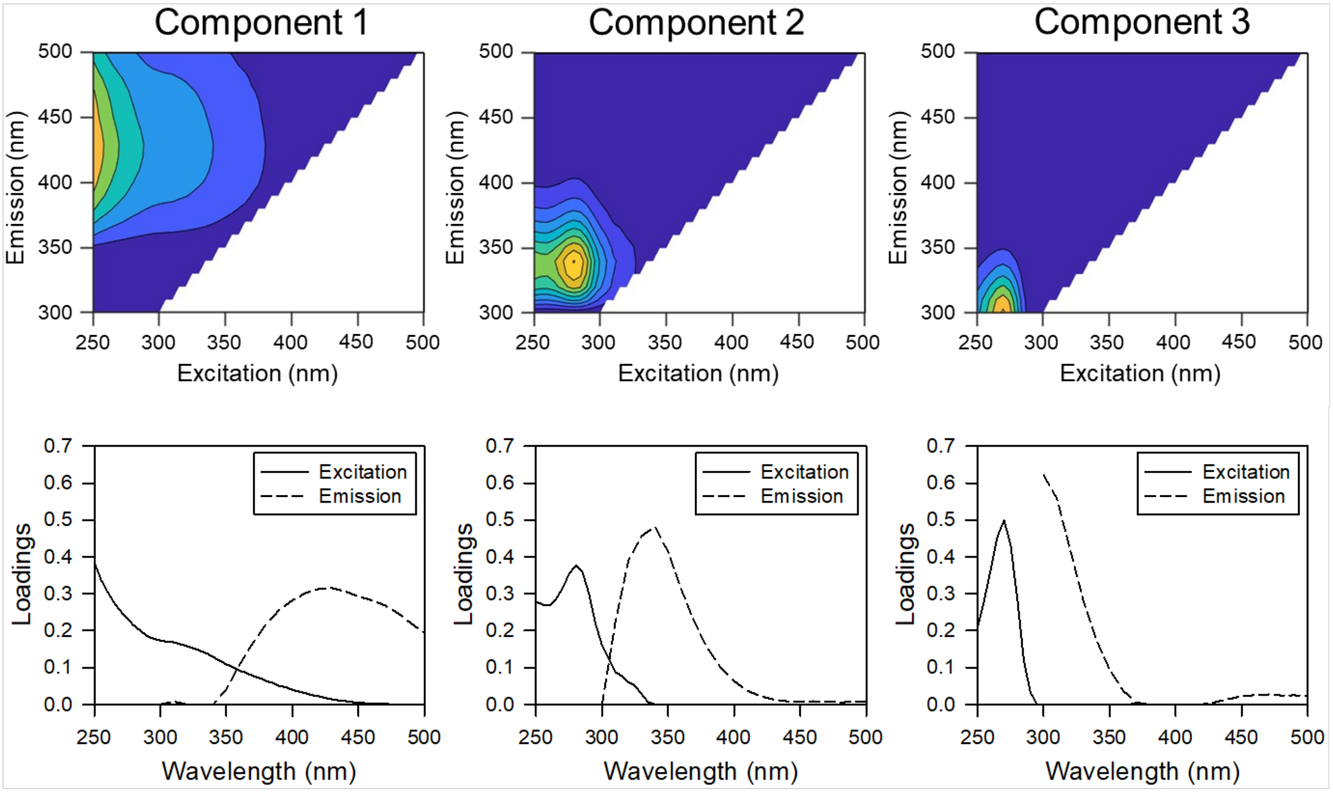


Figure S6. Contour plots of fluorescence EEM spectra and excitation-emission loadings of Component 1 ($FDOM_H$; terrestrial humic-like fluorophore), Component 2 ($FDOM_T$; Tryptophan-like fluorophore), and Component 3 ($FDOM_B$; Tyrosine-like fluorophore) determined using the PARAFAC model in the northern East China Sea.

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