



*Supplement of*

## **Temperature-enhanced effects of iron on Southern Ocean phytoplankton**

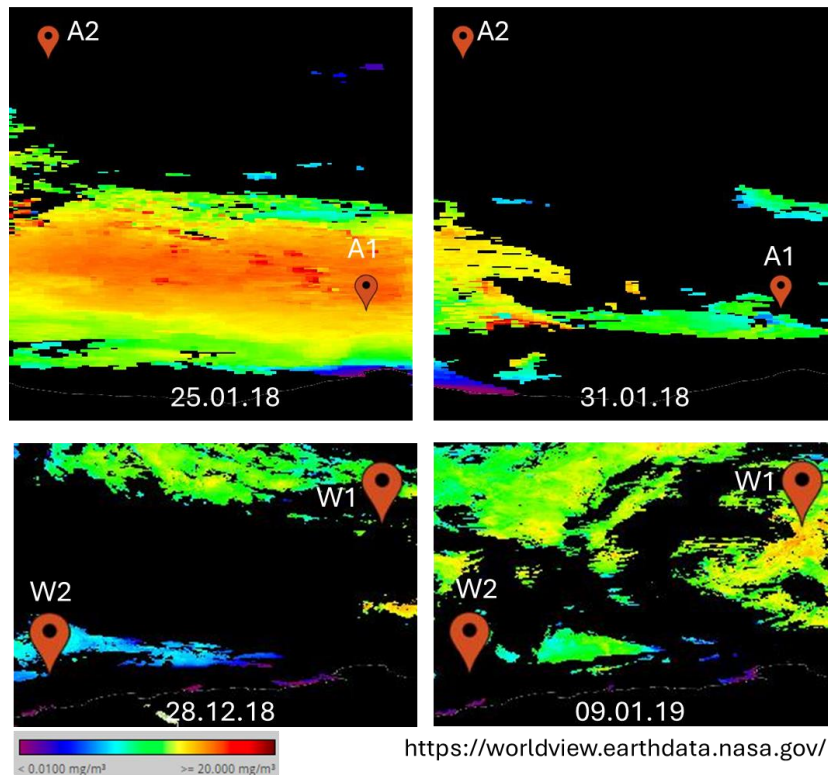
**Charlotte Eich et al.**

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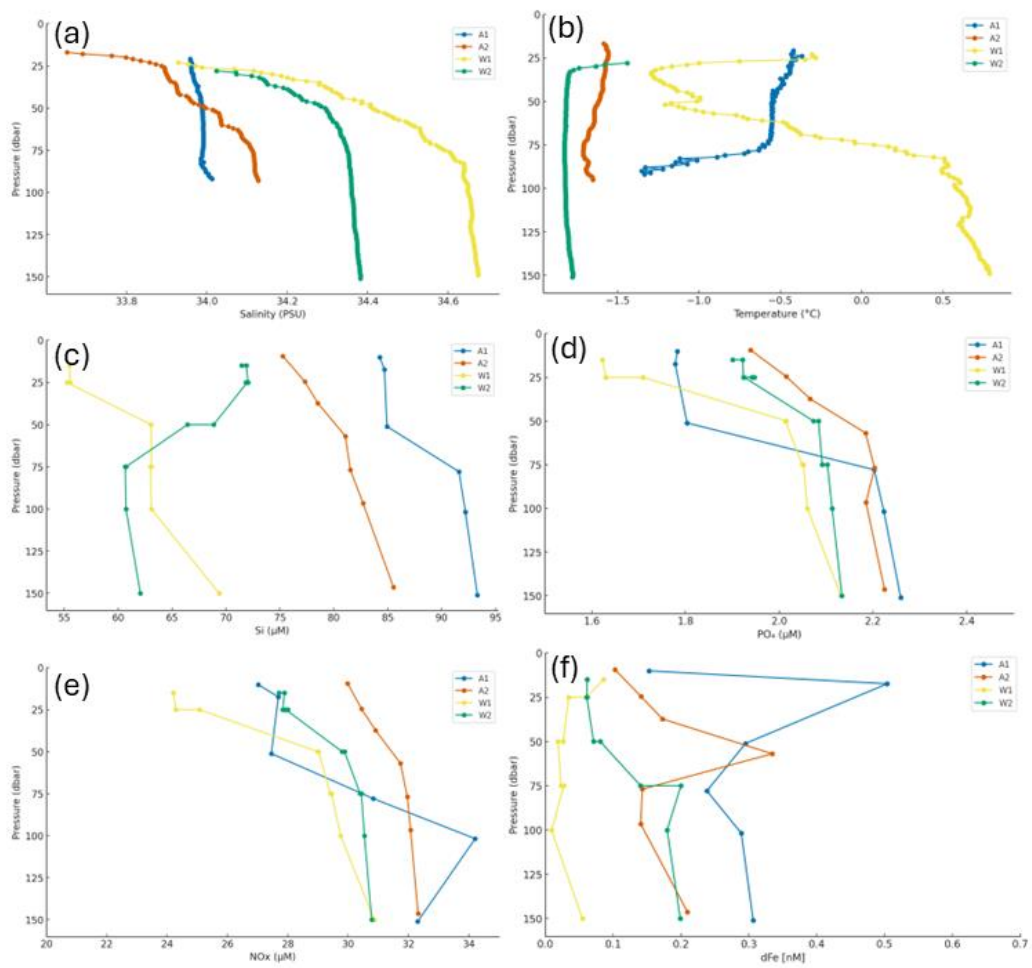
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## **Bioassay set-up**

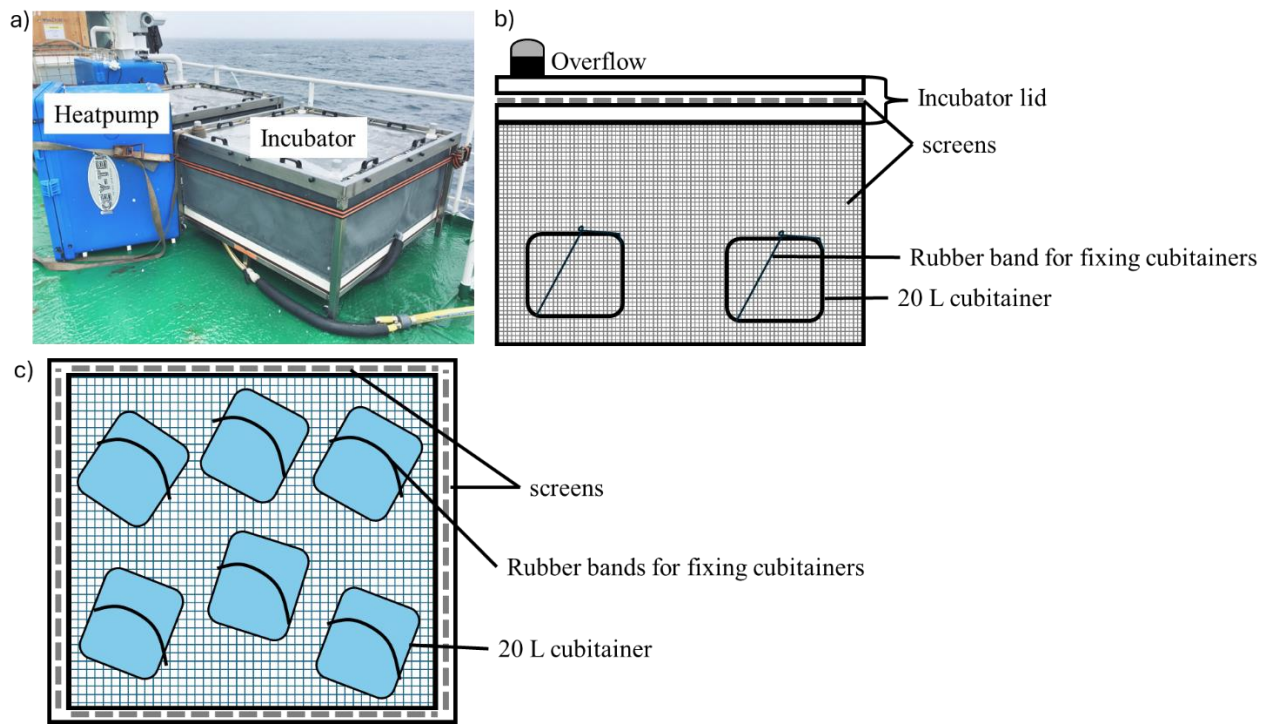
Incubations were performed in custom built on-deck incubators, where temperatures could be maintained stable within a maximum fluctuation of 0.3°C. Incubators consist of a steel frame containing double-walled polycarbonate baths, that can be closed off with air-tight lids (Supplement Fig. S3). Airtightness of lids is ensured by flexible tubing, that can be inflated once the incubators are closed, and the lid is held in position by moveable metal rods. Openings in the lids fitted with PVC pipes with caps allow complete filling of the incubator, i.e. without air bubbles. The temperature was controlled with custom made Aquahort Heat & Chill Water to Water Heatpumps (Aquahort, New Zealand), which needed constant supply of 5°C water, which was provided either from wastewater (ANA08B) or firefighting water (PS117) We monitored temperatures using a calibrated thermometer (VWR, TD20, Radnor, Pennsylvania, USA) and continuously logged using easy log USB (LASCAR electronics, Whiteparish, UK). Light levels were reached by attaching neutral density screens to the lid and sides of the incubator, which were fixed with Velcro and/or placed between the polycarbonate layers (for the lid only). Cubitainers were filled to the top to avoid shear by air-bubbles and fixed with elastic rope in the incubators. Subsampling of the cubitainers was done via a fitted vented PE faucet under trace-metal clean conditions and without introduction of air (cubitainers were continuously pressurised during subsampling).



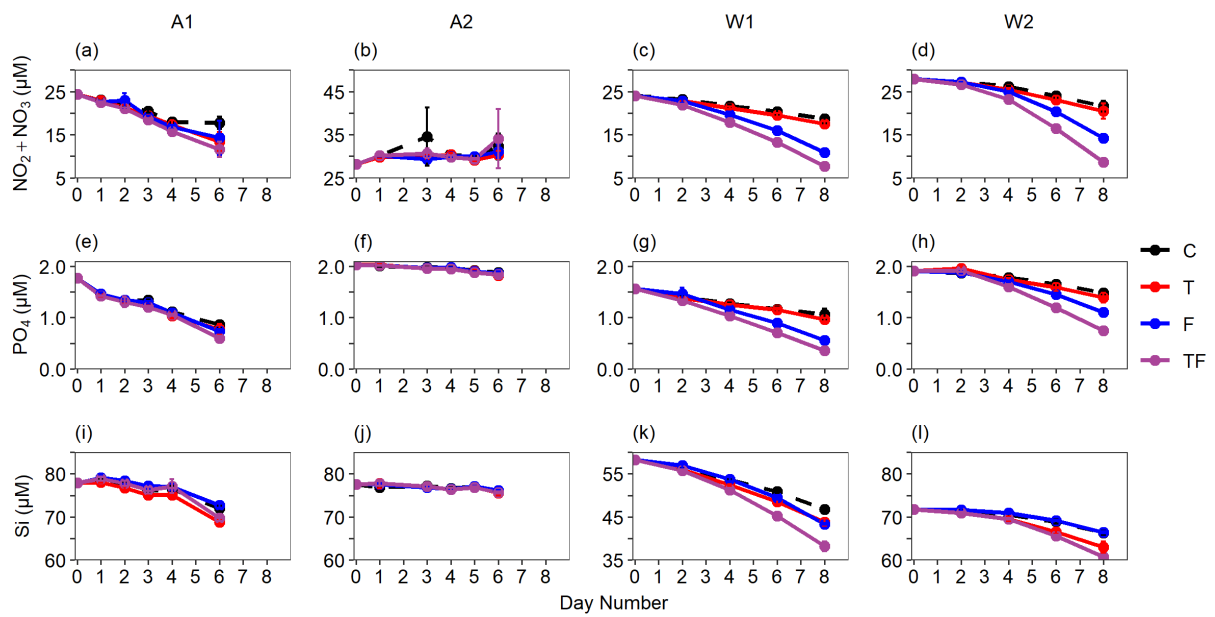
**Supplement Figure S1:** Chlorophyll *a* concentrations at times of sampling, based on NASA worldview (combination of all satellite data available). We acknowledge the use of imagery from the NASA Worldview application (<https://worldview.earthdata.nasa.gov/>), part of the NASA Earth Observing System Data and Information System (EOSDIS).



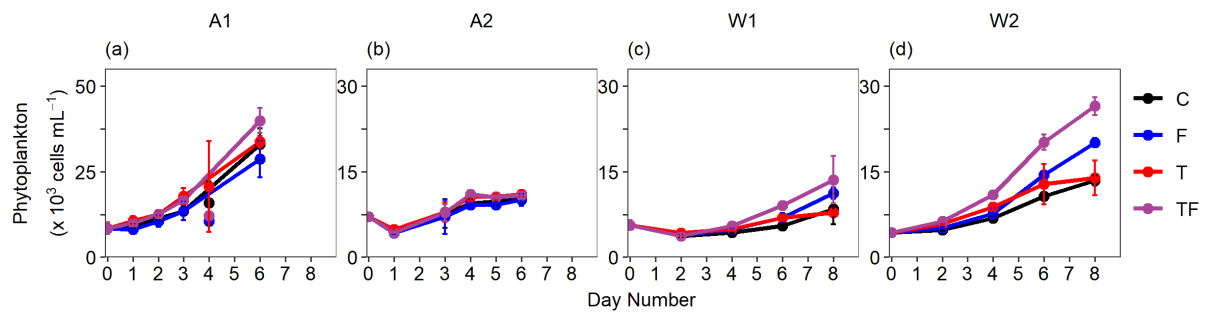
**Supplement Figure S2:** Depth Profiles of Temperature (a), Salinity (b), and Nutrients: (c) Si, (d) PO, (e) NO<sub>x</sub>, and (f) DFe across all experiments. For W1 and W2, nutrient data were unavailable; instead, data from the nearest field station have been used.



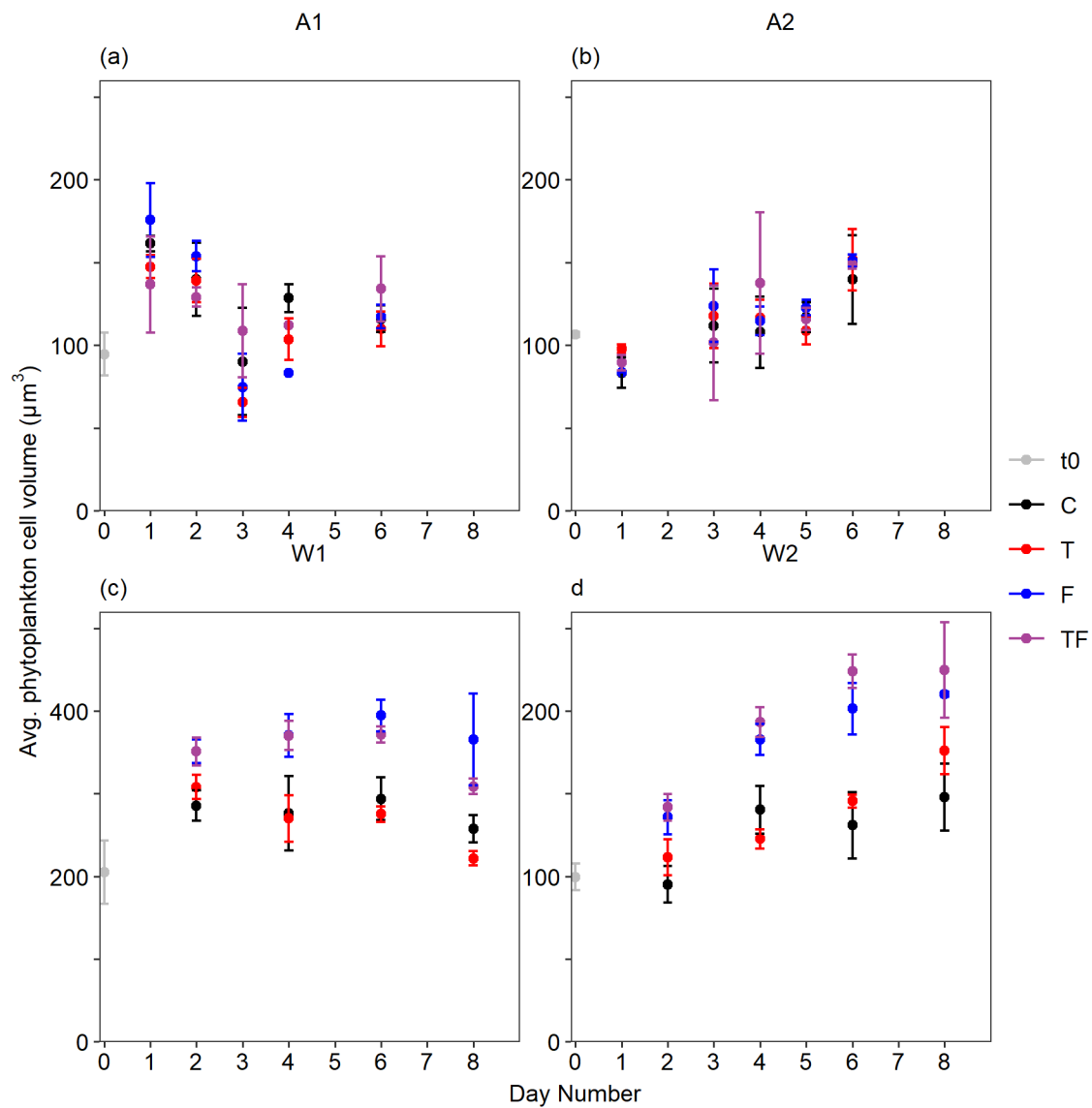
**Supplement Figure S3:** Overview of bioassay setup: On-board setup of incubators and heatpump for temperature control (a), view from the side (b) and view from the top without a lid (c).



**Supplement Figure S4:** Temporal dynamics of the major nutrient concentrations for the Admundsen Sea A1 (a, e, i), A2 (b, f, j) and the Weddel Sea W1 (c, g, k) and W2 (d, h, l) bioassays. The black line represents the control (C) treatment, the red line the temperature (T) treatment, the blue line the iron (F) treatment, and the purple line the combined temperature and iron (TF) treatment. Error bars represent the standard deviation (n = 3); if not visible it is smaller than the symbol. Note that despite the different scale for Si in W1, the tick-marks represent the same concentration changes to facilitate comparison.

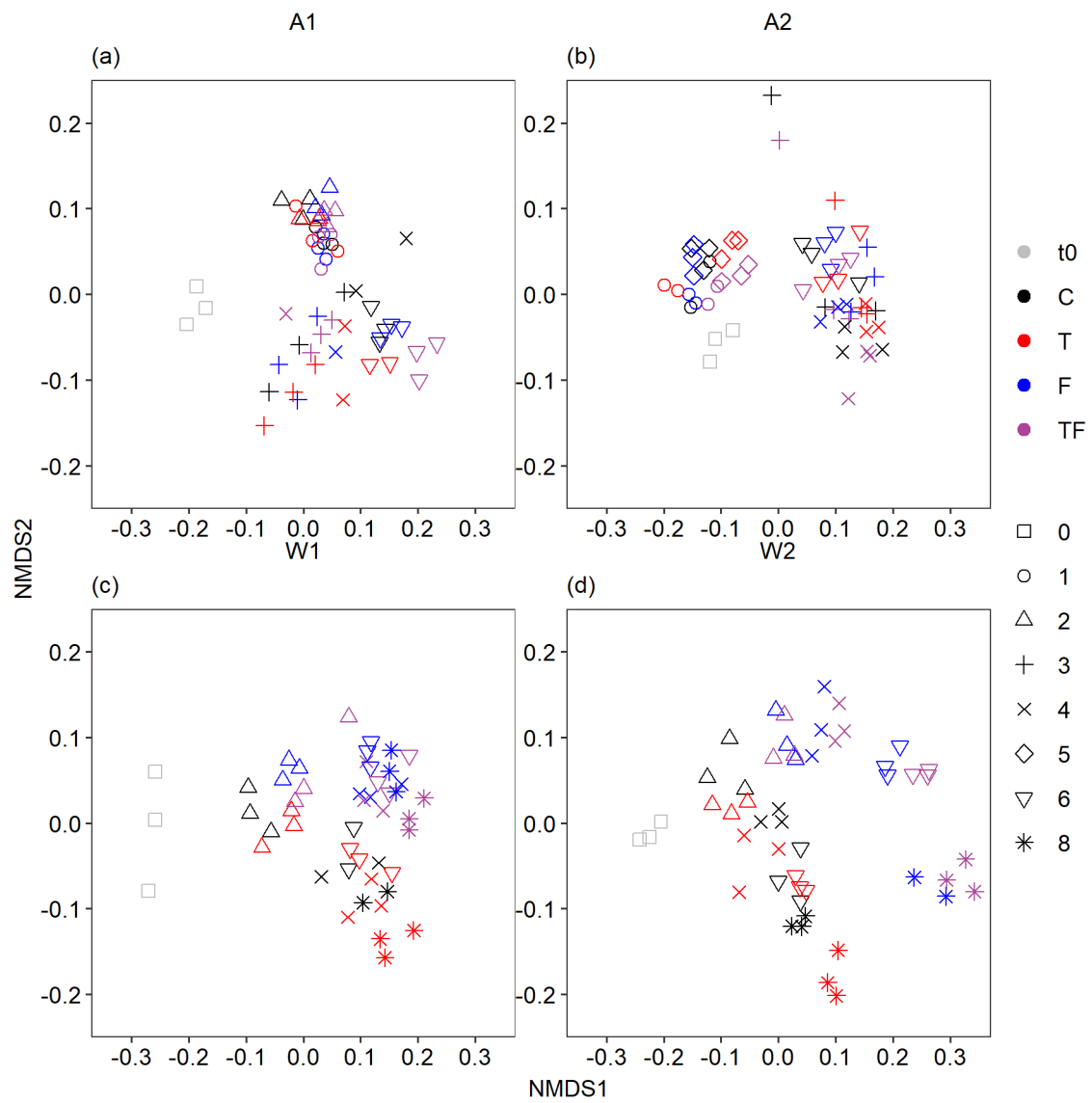


**Supplement Figure S5:** Temporal dynamics of total phytoplankton abundances for the Amundsen Sea A1 and A2 (a, b) and the Weddell Sea W1 and W2 (c, d) bioassays. The black line represents the control (C) treatment, the red line the temperature (T) treatment, the blue line the iron (F) treatment, and the purple line the combined temperature and iron (TF) treatment. Error bars represent the standard deviation ( $n = 3$ ).



**Supplement Figure S6:** Average phytoplankton cell volume. Average biovolume was calculated using total phytoplankton volume assuming spherical cells and dividing by total phytoplankton abundances. (a, b) Amundsen Sea bioassays A1 and A2; (c, d) Weddell Sea bioassays W1 and W2. C = control, T = temperature treatment (+2°C), F = iron addition treatment (+2 nM Fe), TF = combined temperature and iron treatment (+2°C, +2 nM Fe). T0 are conditions at the beginning of the bioassays.





**Supplement Figure S7:** NMDS plot of phytoplankton community compositions for Amundsen Sea bioassays A1 (a) and A2 (b) and Weddell Sea bioassays W1 (c), and W2 (d). The numbers refer to the day of the incubation the phytoplankton community was sampled on.

**Supplement Table S1:** Decrease in dFe concentration after addition to ultrapure water.

Time (hours)	dFe (nM)
0	2.11
0.5	1.93
3	1.75
6	1.72
12	1.83
26	1.45
47	1.19

**Supplement Table S2:** Blanks and limit of detection (LOD) defined as three times the standard deviation of the blank for trace metal samples. N = 20

	MQ blank (pM)	LOD (pM)
Fe	35.0 ± 17	51.9
Mn	1.0 ± 0.9	2.7

**Supplement Table S3:** GEOTRACES community consensus reference materials SAFE D1 and S and GSC and GSP for dissolved trace metal measurements.

	Mn	Fe
	(nM)	(nM)
GSP (n=3)	0.73 ± 0.01	0.13 ± 0.01
<i>Certified value</i>	<i>0.78 ± 0.03</i>	<i>0.16 ± 0.05</i>
GSC (n=3)	2.03 ± 0.04	1.42 ± 0.02
<i>Certified value</i>	<i>2.18 ± 0.08</i>	<i>1.53 ± 0.12</i>
S (n=3)	0.98 ± 0.02	0.10 ± 0.001
<i>Certified value</i>	<i>0.81 ± 0.06</i>	<i>0.10 ± 0.009</i>
D1 (n=3)	0.43 ± 0.005	0.74 ± 0.002
<i>Certified value</i>	<i>NA</i>	<i>0.69 ± 0.040</i>

**Supplement Table S4:** Acid and filters (pmol/vial) of the labile leach (labile) and subsequent total digestion (refractory) for particulate trace metal and POP samples.

	P	Mn	Fe
Acid blank Labile	55.91	0.99	42.69
± 1SD	26.77	0.53	26.42
Acid blank Refractory	174.70	0.34	15.93
± 1SD	213.91	0.19	9.42
Filter blank Labile	93.34	1.39	42.48
± 1SD	80.97	0.26	9.63
Refractory	74.12	0.68	33.06
± 1SD	19.34	0.22	9.00

**Supplement Table S5:** Concentrations and recoveries of Certified Reference Materials for particulate trace metals and POP  $\pm$  1SD. MESS-3 and PACS-2 were solely subjected to the total digestion (total digest). Values are averages of triplicate samples.

	P	Mn	Fe
	(g/100g)	( $\mu$ g/g)	(g/100g)
MESS-3 Certified value	0.12 <sup>a</sup>	324 $\pm$ 1	4.34 $\pm$ 0.11
Total digest	0.11 $\pm$ 0.01	302 $\pm$ 34	3.96 $\pm$ 0.51
Recovery (%)	89 $\pm$ 10	93 $\pm$ 11	91 $\pm$ 12
PACS-2 Certified value	0.096 $\pm$ 0.004	440 $\pm$ 19	4.09 $\pm$ 0.06
Total digest	0.097 $\pm$ 0.010	442 $\pm$ 48	4.26 $\pm$ 0.46
Recovery (%)	101 $\pm$ 10	101 $\pm$ 11	104 $\pm$ 11

*a* Only indicative value available.

**Supplement Table S6:** Final pigment ratios used for CHEMTAX analysis, relative to Chl a. 19-butfuco = 19-butanoloxifucoxanthin, Fuco = fucoxanthin, 19-hexfuco = 19 hexanoloxifucoxanthin, Allox = alloxanthin, Chl\_c3 = chlorophyll c3, Chl\_b = chlorophyll b.

Group	Peridinin	19-butfuco	Fuco	19-hexfuco	Allox	Chl_c3	Chl_b
Chlorophytes							0.77
Dinoflagellates	0.69						
Cryptophytes					0.29		
Haptophytes		0.01	0.30	0.65		0.17	
Pelagophytes		0.10	0.30	0.10		0.02	
Diatoms			0.62				

**Supplement Table S7:** Average cell size of the phytoplankton populations discriminated by flow cytometry.

Population	Diameter ( $\mu\text{m}$ )
Phyto 1	1.2
Phyto 2	2.0
Phyto 3	2.0
Phyto 4	2.5
Phyto 5	3.0
Phyto 6	3.0
Phyto 7	3.2
Phyto 8	3.7
Phyto 9	4.3
Phyto 10	4.9
Phyto 11	5.2
Phyto 12	5.2
Phyto 13	5.5
Phyto 14	6.0
Phyto 15	6.7
Phyto 16	7.1
Phyto 17	7.3
Phyto 18	7.8
Phyto 19	8.1
Phyto 20	8.8
Phyto 21	10.4
Phyto 22	13.3
Phyto 23	15.4
Phyto 24	19.0
Phyto 25	19.8



**Supplement Table S8:** Results of the Scheirer-Ray-Hare-Test for dissolved (d) and particulate (p) metals. Analysis was performed based on data from the last day of the incubation (t6 for Amundsen Sea and t8 for Weddell Sea bioassays). Fe: iron, Mn: manganese, Df: degrees of freedom, Sum Sq: sum of squares, H: H-test statistic,  $\eta^2$ : eta-squared (effect size), Fe: iron addition, temp: temperature increase, Fe x temperature: interaction of iron addition and temperature increase.

Bio	variable	d <sup>56</sup> Fe					d <sup>57</sup> Fe					dMn				
		Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
A1	Fe	1	0.0	0.00	0.95	0.00						1	10.0	0.93	0.34	0.09
A1	temp	1	15.2	1.39	0.24	0.14						1	56.9	5.27	0.02	0.51
A1	Fe x temp	1	3.1	0.29	0.59	0.03						1	14.5	1.34	0.25	0.13
A1	Residuals	7	90.3									7	30.7			
A2	Fe	1	66.2	7.26	0.01	0.77						1	0.8	0.06	0.81	0.01
A2	temp	1	3.8	0.41	0.52	0.04						1	96.3	7.65	0.01	0.70
A2	Fe x temp	1	3.8	0.41	0.52	0.04						1	0.1	0.01	0.94	0.00
A2	Residuals	6	12.0									8	41.3			
W1	Fe	1	6.3	0.70	0.40	0.07	1	77.3	7.19	0.01	0.76	1	6.2	0.68	0.41	0.07
W1	temp	1	23.4	2.57	0.11	0.27	1	5.9	0.55	0.46	0.06	1	47.3	5.17	0.02	0.50
W1	Fe x temp	1	8.4	0.93	0.34	0.10	1	7.8	0.72	0.39	0.08	1	7.8	0.85	0.36	0.08
W1	Residuals	6	47.6				7	11.3				7	33.1			
W2	Fe	1	28.0	2.63	0.10	0.25	1	79.5	7.99	0.00	0.82	1	2.1	0.17	0.68	0.02
W2	temp	1	22.5	2.12	0.15	0.20	1	1.5	0.15	0.70	0.02	1	0.8	0.06	0.81	0.01
W2	Fe x temp	1	7.0	0.66	0.42	0.06	1	0.2	0.02	0.88	0.00	1	5.3	0.43	0.51	0.04
W2	Residuals	7	53.5				7	15.3				8	128.8			

Bio	variable	p <sup>56</sup> Fe					p <sup>57</sup> Fe					pMn				
		Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
A1	Fe	1	60.0	6.55	0.01	0.73						1	51.6	4.71	0.03	0.50
A1	temp	1	2.5	0.27	0.60	0.03						1	12.2	1.12	0.29	0.12
A1	Fe x temp	1	1.7	0.18	0.67	0.02						1	3.1	0.29	0.59	0.03
A1	Residuals	6	18.3									7	36.8			
W1	Fe	1	17.9	2.38	0.12	0.27	1	84.4	7.68	0.01	0.75	1	59.7	5.51	0.02	0.52
W1	temp	1	21.6	2.87	0.09	0.33	1	2.3	0.21	0.65	0.02	1	24.9	2.30	0.13	0.22
W1	Fe x temp	1	6.8	0.90	0.34	0.10	1	1.9	0.17	0.68	0.02	1	3.1	0.29	0.59	0.03
W1	Residuals	5	19.2				7	23.3				7	27.7			
W2	Fe	1	18.0	3.00	0.08	0.43	1	62.5	6.82	0.01	0.76	1	21.3	1.67	0.20	0.15
W2	temp	1	18.0	3.00	0.08	0.43	1	6.7	0.73	0.39	0.08	1	18.8	1.47	0.23	0.13
W2	Fe x temp	1	0.0	0.00	1.00	0.00	1	1.7	0.18	0.67	0.02	1	6.8	0.53	0.47	0.05
W2	Residuals	4	6.0				6	11.7				8	93.7			

**Supplement Table S9:** Results of the Scheirer-Ray-Hare-Test for macronutrients. Analysis was performed based on data from the last day of the incubation (t6 for Amundsen Sea and t8 for Weddell Sea bioassays). Si: silicate, PO<sub>4</sub>: phosphate, NO<sub>x</sub>: nitrogen (NO<sub>2</sub> + NO<sub>3</sub>). Df: degrees of freedom, Sum Sq: sum of squares, H: H-test statistic,  $\eta^2$ : eta-squared (effect size), Fe: iron addition, temp: temperature increase, Fe x temperature: interaction of iron addition and temperature increase.

Bio	variable	Si					PO <sub>4</sub>					NO <sub>x</sub>				
		Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
A1	Fe	1	8.3	0.64	0.42	0.06	1	75.0	5.79	0.02	0.53	1	33.3	2.56	0.11	0.23
A1	temp	1	108.0	8.31	0.00	0.76	1	33.3	2.57	0.11	0.23	1	48.0	3.69	0.05	0.34
A1	Fe x temp	1	0.0	0.00	1.00	0.00	1	1.3	0.10	0.75	0.01	1	0.3	0.03	0.87	0.00
A1	Residuals	8	26.7				8	32.8				8	61.3			
A2	Fe	1	0.0	0.00	1.00	0.00	1	0.1	0.01	0.94	0.00	1	5.3	0.41	0.52	0.04
A2	temp	1	75.0	5.77	0.02	0.52	1	65.3	5.15	0.02	0.47	1	1.3	0.10	0.75	0.01
A2	Fe x temp	1	1.3	0.10	0.75	0.01	1	18.8	1.48	0.22	0.13	1	8.3	0.64	0.42	0.06
A2	Residuals	8	66.7				8	55.3				8	128.0			
W1	Fe	1	61.6	5.60	0.02	0.52	1	89.0	8.09	0.00	0.76	1	90.1	8.19	0.00	0.77
W1	temp	1	38.5	3.50	0.06	0.32	1	15.6	1.42	0.23	0.13	1	20.8	1.89	0.17	0.18
W1	Fe x temp	1	0.2	0.02	0.90	0.00	1	1.2	0.11	0.74	0.01	1	0.2	0.02	0.90	0.00
W1	Residuals	7	18.5				7	10.7				7	6.5			
W2	Fe	1	5.3	0.41	0.52	0.04	1	108.0	8.34	0.00	0.76	1	108.0	8.31	0.00	0.76
W2	temp	1	108.0	8.31	0.00	0.76	1	24.1	1.86	0.17	0.17	1	16.3	1.26	0.26	0.11
W2	Fe x temp	1	8.3	0.64	0.42	0.06	1	0.1	0.01	0.94	0.00	1	1.3	0.10	0.75	0.01
W2	Residuals	8	21.3				8	10.3				8	17.3			

**Supplement Table S10:** Results of the Scheirer-Ray-Hare-Test for silicate to nitrogen (Si:N) and silicate to phosphorous (Si:P) ratios. Df: degrees of freedom, Sum Sq: sum of squares, H: H-test statistic,  $\eta^2$ : eta-squared (effect size), Fe: iron addition, temp: temperature increase, Fe x temperature: interaction of iron addition and temperature increase. Analysis was performed based on data from the last day of the incubation (t6 for Amundsen Sea and t8 for Weddell Sea bioassays).

Bio	variable	Si:N					Si:P				
		Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
A1	Fe	1	40.3	3.10	0.08	0.28	1	85.3	6.56	0.01	0.60
A1	temp	1	40.3	3.10	0.08	0.28	1	21.3	1.64	0.20	0.15
A1	Fe x temp	1	0.3	0.03	0.87	0.00	1	3.0	0.23	0.63	0.02
A1	Residuals	8	62.0				8	33.3			
A2	Fe	1	3.0	0.23	0.63	0.02	1	0.3	0.03	0.87	0.00
A2	temp	1	0.0	0.00	1.00	0.00	1	40.3	3.10	0.08	0.28
A2	Fe x temp	1	5.3	0.41	0.52	0.04	1	27.0	2.08	0.15	0.19
A2	Residuals	8	134.7				8	75.3			
W1	Fe	1	86.7	7.88	0.00	0.76	1	86.7	7.88	0.00	0.76
W1	temp	1	7.5	0.68	0.41	0.07	1	7.5	0.68	0.41	0.07
W1	Fe x temp	1	6.0	0.55	0.46	0.05	1	6.0	0.55	0.46	0.05
W1	Residuals	7	14.0				7	14.0			
W2	Fe	1	108.0	8.31	0.00	0.76	1	108.0	8.31	0.00	0.76
W2	temp	1	8.3	0.64	0.42	0.06	1	3.0	0.23	0.63	0.02
W2	Fe x temp	1	5.3	0.41	0.52	0.04	1	12.0	0.92	0.34	0.08
W2	Residuals	8	21.3				8	20.0			

**Supplement Table S11:** Results of the Scheirer-Ray-Hare-Test for particulate metal to particulate (p) organic phosphorus (POP) ratios. Fe: iron, Mn: manganese, Zn:zinc, Cu: copper, Cd:cadmium . Df: degrees of freedom, Sum Sq: sum of squares, H: H-test statistic,  $\eta^2$ : eta-squared (effect size), Fe: iron addition, temp: temperature increase, Fe x temperature: interaction of iron addition and temperature increase. Analysis was performed based on data from the last day of the incubation (t6 for Amundsen Sea and t8 for Weddell Sea bioassays).

Bio	variable	p <sup>56</sup> Fe:POP					p <sup>57</sup> Fe:POP					dMn:POP				
		Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
A1	Fe	1	60.0	6.55	0.01	0.73						1	78.9	7.18	0.01	0.74
A1	temp	1	4.9	0.53	0.46	0.06						1	2.3	0.21	0.65	0.02
A1	Fe x temp	1	3.3	0.36	0.55	0.04						1	1.9	0.17	0.68	0.02
A1	Residuals	6	14.3									7	23.3			
W1	Fe	1	0.7	0.10	0.75	0.01	1	83.3	7.58	0.01	0.75	1	0.1	0.01	0.94	0.00
W1	temp	1	29.5	3.94	0.05	0.49	1	0.8	0.08	0.78	0.01	1	5.9	0.54	0.46	0.05
W1	Fe x temp	1	26.0	3.46	0.06	0.43	1	4.2	0.38	0.54	0.04	1	68.9	6.26	0.01	0.63
W1	Residuals	5	4.5				7	22.5				7	35.2			
W2	Fe	1	32.0	5.33	0.02	0.76	1	62.5	6.82	0.01	0.76	1	108.0	8.31	0.00	0.76
W2	temp	1	8.0	1.33	0.25	0.19	1	1.7	0.18	0.67	0.02	1	3.0	0.23	0.63	0.02
W2	Fe x temp	1	0.0	0.00	1.00	0.00	1	6.7	0.73	0.39	0.08	1	5.3	0.41	0.52	0.04
W2	Residuals	4	2.0				6	11.7				8	26.7			

Bio	variable	pZn:POP					pCu:POP					pCd:POP				
		Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
A1	Fe	1	4.3	0.39	0.53	0.04	1	85.6	7.78	0.01	0.76	1	0.8	0.08	0.78	0.01
A1	temp	1	11.6	1.06	0.30	0.11	1	4.5	0.41	0.52	0.04	1	9.6	0.88	0.35	0.09
A1	Fe x temp	1	0.0	0.00	0.97	0.00	1	9.8	0.89	0.35	0.09	1	20.2	1.83	0.18	0.18
A1	Residuals	7	92.5				7	13.2				7	79.8			
W1	Fe	1	70.5	6.41	0.01	0.65	1	26.7	2.91	0.09	0.32	1	85.6	7.78	0.01	0.76
W1	temp	1	0.1	0.01	0.91	0.00	1	0.9	0.10	0.75	0.01	1	4.5	0.41	0.52	0.04
W1	Fe x temp	1	8.2	0.74	0.39	0.08	1	35.3	3.85	0.05	0.43	1	9.8	0.89	0.35	0.09
W1	Residuals	7	29.8				6	19.7				7	13.2			
W2	Fe	1	108.0	8.31	0.00	0.76	1	75.7	6.89	0.01	0.73	1	42.4	3.85	0.05	0.39
W2	temp	1	1.3	0.10	0.75	0.01	1	11.2	1.02	0.31	0.11	1	1.3	0.12	0.73	0.01
W2	Fe x temp	1	0.3	0.03	0.87	0.00	1	0.5	0.04	0.84	0.00	1	3.1	0.28	0.59	0.03
W2	Residuals	8	33.3				7	15.8				7	61.2			

**Supplement Table S12:** Results of the Scheirer-Ray-Hare-Test for  $F_v/F_m$ : photosynthetic efficiency of phytoplankton, Total Phyto: total phytoplankton abundances based on flow cytometry, Bacteria: total bacterial abundances based on flow cytometry. Df: degrees of freedom, Sum Sq: sum of squares, H: H-test statistic,  $\eta^2$ : eta-squared (effect size), Fe: iron addition, temp: temperature increase, Fe x temperature: interaction of iron addition and temperature increase. Analysis was performed based on data from the last day of the incubation (t6 for Amundsen Sea and t8 for Weddell Sea bioassays).

Bio	variable	$F_v/F_m$					Total Phyto					Bacteria				
		Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
A1	Fe	1	108.0	8.46	0.00	0.77	1	1.5	0.13	0.71	0.01	1	2.0	0.18	0.67	0.02
A1	temp	1	5.3	0.42	0.52	0.04	1	34.8	3.17	0.08	0.32	1	3.8	0.34	0.56	0.03
A1	Fe x temp	1	3.0	0.23	0.63	0.02	1	39.2	3.56	0.06	0.36	1	34.2	3.11	0.08	0.31
A1	Residuals	8	24.2				7	32.7				7	70.5			
A2	Fe	1	96.3	7.52	0.01	0.68	1	8.3	0.64	0.42	0.06	1	0.6	0.06	0.81	0.01
A2	temp	1	0.3	0.03	0.87	0.00	1	21.3	1.64	0.20	0.15	1	17.1	1.56	0.21	0.16
A2	Fe x temp	1	3.0	0.23	0.63	0.02	1	0.0	0.00	1.00	0.00	1	4.7	0.43	0.51	0.04
A2	Residuals	8	41.3				8	113.3				7	86.7			
W1	Fe	1	73.6	6.69	0.01	0.73	1	61.6	5.60	0.02	0.56	1	62.6	5.69	0.02	0.57
W1	temp	1	20.8	1.89	0.17	0.21	1	0.0	0.00	0.96	0.00	1	0.6	0.06	0.81	0.01
W1	Fe x temp	1	0.2	0.02	0.90	0.00	1	2.7	0.24	0.62	0.02	1	2.2	0.20	0.65	0.02
W1	Residuals	7	6.5				7	45.3				7	45.2			
W2	Fe	1	108.0	8.34	0.00	0.76	1	77.9	7.08	0.01	0.74	1	65.3	5.03	0.02	0.46
W2	temp	1	14.1	1.09	0.30	0.10	1	4.5	0.41	0.52	0.04	1	5.3	0.41	0.52	0.04
W2	Fe x temp	1	2.1	0.16	0.69	0.01	1	3.1	0.28	0.59	0.03	1	27.0	2.08	0.15	0.19
W2	Residuals	8	18.3				7	19.8				8	45.3			

**Supplement Table S13:** Results of the Scheirer-Ray-Hare-Test for particulate organic carbon (POC), total Chl *a*, < 20  $\mu\text{m}$  Chl *a* and total Chl *a*:POC ratio. Df: degrees of freedom, Sum Sq: sum of squares, H: H-test statistic,  $\eta^2$ : eta-squared (effect size), Fe: iron addition, temp: temperature increase, Fe x temperature: interaction of iron addition and temperature increase. Analysis was performed based on data from the last day of the incubation (t6 for Amundsen Sea and t8 for Weddell Sea bioassays).

Bio	variable	POC					total Chl <i>a</i>				
		Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
A1	Fe	1	5.3	0.41	0.52	0.04	1	75.0	5.77	0.02	0.52
A1	temp	1	0.3	0.03	0.87	0.00	1	27.0	2.08	0.15	0.19
A1	Fe x temp	1	12.0	0.92	0.34	0.08	1	3.0	0.23	0.63	0.02
A1	Residuals	8	125.3				8	38.0			
A2	Fe	1	3.0	0.23	0.63	0.02	1	8.6	3.44	0.06	0.77
A2	temp	1	5.3	0.41	0.52	0.04	1	1.9	0.77	0.38	0.17
A2	Fe x temp	1	0.0	0.00	1.00	0.00	1	0.1	0.03	0.87	0.01
A2	Residuals	8	134.7				1	0.5			
W1	Fe	1	90.1	8.19	0.00	0.77	1	72.3	7.88	0.00	0.76
W1	temp	1	20.8	1.89	0.17	0.18	1	16.0	1.75	0.19	0.17
W1	Fe x temp	1	0.2	0.02	0.90	0.00	1	0.5	0.05	0.82	0.01
W1	Residuals	7	6.5				6	6.0			
W2	Fe	1	108.0	8.31	0.00	0.76	1	96.3	7.41	0.01	0.67
W2	temp	1	21.3	1.64	0.20	0.15	1	12.0	0.92	0.34	0.08
W2	Fe x temp	1	0.3	0.03	0.87	0.00	1	5.3	0.41	0.52	0.04
W2	Residuals	8	13.3				8	29.3			

Bio	variable	< 20 $\mu\text{m}$ Chl <i>a</i>					total Chl <i>a</i> :POC				
		Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
A1	Fe	1	52.1	4.02	0.04	0.37	1	85.3	6.56	0.01	0.60
A1	temp	1	24.1	1.86	0.17	0.17	1	33.3	2.56	0.11	0.23
A1	Fe x temp	1	1.3	0.10	0.75	0.01	1	0.3	0.03	0.87	0.00
A1	Residuals	8	65.0				8	24.0			
A2	Fe	1	1.9	0.77	0.38	0.17	1	8.6	3.44	0.06	0.77
A2	temp	1	8.6	3.44	0.06	0.77	1	1.9	0.77	0.38	0.17
A2	Fe x temp	1	0.1	0.03	0.87	0.01	1	0.1	0.03	0.87	0.01
A2	Residuals	1	0.5				1	0.5			
W1	Fe	1	60.0	6.55	0.01	0.73	1	66.7	7.28	0.01	0.75
W1	temp	1	0.1	0.01	0.92	0.00	1	7.1	0.78	0.38	0.08
W1	Fe x temp	1	5.4	0.59	0.44	0.07	1	0.1	0.01	0.94	0.00
W1	Residuals	6	17.0				6	15.3			
W2	Fe	1	96.3	7.41	0.01	0.67	1	56.3	4.33	0.04	0.39
W2	temp	1	8.3	0.64	0.42	0.06	1	0.3	0.03	0.87	0.00
W2	Fe x temp	1	8.3	0.64	0.42	0.06	1	56.3	4.33	0.04	0.39
W2	Residuals	8	30.0				8	30.0			

**Supplement Table S14:** Significant indicator species analysis results. Bio: bioassay, Phyto: Phytoplankton group, ISIA: indicator species analysis results based on abundances, ISA R: indicator species analysis results based on relative abundances, ISA C: indicator species analysis results based on phytoplankton carbon. Letters stand for the respective treatments where indicator species analysis was significant (C: control, F: iron addition treatment, T: temperature treatment, TF: iron-temperature treatment). Only results for the final day of incubations (t6 for Amundsen Sea and t8 for Weddell Sea bioassays) are reported, except when noted otherwise.

Bio	Phyto	ISA A	ISA R	ISA C
A1	3			TF
A1	16		C, F	C
A1	19		T, TF	T, TF
A1	23		C & T	C & T
A1	24		TF	
A2	19			T, TF
A2	20			C, F
A2	22			T, TF
W1	1	F, TF		F, TF
W1	3		T	
W1	4		C, T	
W1	5	F, TF	F, TF	F, TF
W1	6		C, F, TF	
W1	8	TF		TF
W1	9		C, T, TF	
W1	12	F, TF		F, TF
W1	16	F, TF	F, TF	F, TF
W1	17	F, TF		F, TF
W1	18	F, TF	F, TF	F, TF
W1	20		C, T	
W1	21	F, TF	F, TF	F, TF
W1	22	F, TF		F, TF
W1	23	F, TF	F, TF	F, TF
W1	24	F, TF		F, TF
W2	1		C, T	
W2	2		C, T	
W2	3	C, F		C, F
W2	4	F (t6), TF (t6)	F (t6), TF (t6)	F (t6), TF (t6)
W2	6	TF		TF
W2	7	C, T	C, T	C, T
W2	8	TF	T	TF
W2	12	TF		TF
W2	13	C, T	C, T	C, T
W2	16	F, TF	F, TF	F, TF
W2	17	F, TF	F, TF	F, TF
W2	20		C, T	
W2	22	TF, T		TF, T
W2	23	TF		TF
W2	24	F, TF	F, TF	F, TF
W2	25	F, TF		F, TF

**Supplement Table S15:** Results of the Scheirer-Ray-Hare-Test for flow-cytometry based group specific net growth rates of phytoplankton in bioassay A1. Df: degrees of freedom, Sum Sq: sum of squares, H: H-test statistic,  $\eta^2$ : eta-squared (effect size), Fe: iron addition, temp: temperature increase, Fe x temperature: interaction of iron addition and temperature increase.

variable	Phyto 3					Phyto 8					Phyto 16				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	0.0	0.00	1.00	0.00	1	5.3	0.41	0.52	0.04	1	0.3	0.03	0.87	0.00
temp	1	75.0	5.77	0.02	0.52	1	8.3	0.64	0.42	0.06	1	56.3	4.33	0.04	0.39
Fe x temp	1	12.0	0.92	0.34	0.08	1	21.3	1.64	0.20	0.15	1	16.3	1.26	0.26	0.11
Residuals	8	56.0				8	108.0				8	70.0			
variable	Phyto 17					Phyto 19					Phyto 20				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	56.3	4.33	0.04	0.39	1	8.3	0.64	0.42	0.06	1	5.3	0.41	0.52	0.04
temp	1	1.3	0.10	0.75	0.01	1	56.3	4.33	0.04	0.39	1	16.3	1.26	0.26	0.11
Fe x temp	1	5.3	0.41	0.52	0.04	1	27.0	2.08	0.15	0.19	1	0.0	0.00	1.00	0.00
Residuals	8	80.0				8	51.3				8	121.3			
variable	Phyto 22					Phyto 23					Phyto 24				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	3.0	0.23	0.63	0.02	1	40.3	3.10	0.08	0.28	1	40.8	3.71	0.05	0.38
temp	1	21.3	1.64	0.20	0.15	1	0.0	0.00	1.00	0.00	1	5.6	0.51	0.47	0.05
Fe x temp	1	85.3	6.56	0.01	0.60	1	5.3	0.41	0.52	0.04	1	28.2	2.56	0.11	0.26
Residuals	8	33.3				8	97.3				7	31.8			



**Supplement Table S16:** Results of the Scheirer-Ray-Hare-Test for flow-cytometry based group specific net growth rates of phytoplankton in bioassay A2. Df: degrees of freedom, Sum Sq: sum of squares, H: H-test statistic,  $\eta^2$ : eta-squared (effect size), Fe: iron addition, temp: temperature increase, Fe x temperature: interaction of iron addition and temperature increase.

variable	Phyto 3					Phyto 4					Phyto 8				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	2.0	0.33	0.56	0.05	1	1.3	0.10	0.75	0.01	1	0.0	0.00	1.00	0.00
temp	1	4.5	0.75	0.39	0.11	1	27.0	2.08	0.15	0.19	1	0.0	0.00	1.00	0.00
Fe x temp	1	0.5	0.08	0.77	0.01	1	12.0	0.92	0.34	0.08	1	2.0	0.33	0.56	0.05
Residuals	4	35.0				8	102.7				4	40.0			
variable	Phyto 11					Phyto 16					Phyto 17				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	0.3	0.03	0.87	0.00	1	75.0	5.77	0.02	0.52	1	75.0	5.77	0.02	0.52
temp	1	16.3	1.26	0.26	0.11	1	3.0	0.23	0.63	0.02	1	3.0	0.23	0.63	0.02
Fe x temp	1	0.3	0.03	0.87	0.00	1	3.0	0.23	0.63	0.02	1	0.3	0.03	0.87	0.00
Residuals	8	126.0				8	62.0				8	64.7			
variable	Phyto 19					Phyto 22					Phyto 24				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	2.0	0.33	0.56	0.05	1	4.5	0.75	0.39	0.11	1	5.3	0.41	0.52	0.04
temp	1	2.0	0.33	0.56	0.05	1	0.5	0.08	0.77	0.01	1	21.3	1.64	0.20	0.15
Fe x temp	1	2.0	0.33	0.56	0.05	1	0.0	0.00	1.00	0.00	1	8.3	0.64	0.42	0.06
Residuals	4	36.0				4	37.0				8	108.0			

**Supplement Table S17:** Results of the Scheirer-Ray-Hare-Test for flow-cytometry based group specific net growth rates of phytoplankton in bioassay W1. Df: degrees of freedom, Sum Sq: sum of squares, H: H-test statistic,  $\eta^2$ : eta-squared (effect size), Fe: iron addition, temp: temperature increase, Fe x temperature: interaction of iron addition and temperature increase.

variable	Phyto 1					Phyto 3					Phyto 4				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	82.2	7.48	0.01	0.75	1	28.4	3.10	0.08	0.34	1	28.0	2.55	0.11	0.26
temp	1	0.1	0.01	0.93	0.00	1	1.8	0.19	0.66	0.02	1	1.6	0.15	0.70	0.02
Fe x temp	1	0.9	0.08	0.77	0.01	1	0.1	0.01	0.94	0.00	1	0.7	0.06	0.81	0.01
Residuals	7	26.5				6	54.0				7	78.0			
variable	Phyto 8					Phyto 9					Phyto 16				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	83.3	7.58	0.01	0.75	1	1.6	0.15	0.70	0.01	1	81.1	7.38	0.01	0.75
temp	1	0.8	0.08	0.78	0.01	1	45.6	4.15	0.04	0.41	1	0.1	0.01	0.93	0.00
Fe x temp	1	4.2	0.38	0.54	0.04	1	16.7	1.52	0.22	0.15	1	11.6	1.05	0.31	0.11
Residuals	7	22.5				7	47.3				7	15.8			
variable	Phyto 17					Phyto 18					Phyto 20				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	84.4	7.68	0.01	0.75	1	2.3	0.21	0.65	0.02	1	33.4	3.04	0.08	0.31
temp	1	2.3	0.21	0.65	0.02	1	10.0	0.91	0.34	0.09	1	5.9	0.54	0.46	0.06
Fe x temp	1	1.9	0.17	0.68	0.02	1	75.9	6.90	0.01	0.68	1	15.6	1.42	0.23	0.15
Residuals	7	23.3				7	22.7				7	51.8			
variable	Phyto 21					Phyto 22					Phyto 23				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	82.2	7.48	0.01	0.75	1	68.5	6.23	0.01	0.64	1	67.5	6.14	0.01	0.64
temp	1	0.1	0.01	0.93	0.00	1	2.5	0.23	0.63	0.02	1	4.8	0.44	0.51	0.05
Fe x temp	1	0.1	0.01	0.93	0.00	1	9.0	0.81	0.37	0.08	1	6.0	0.55	0.46	0.06
Residuals	7	27.3				7	26.7				7	27.3			
variable	Phyto 24					Phyto 25									
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$					
Fe	1	56.0	5.09	0.02	0.54	1	28.0	2.55	0.11	0.26					
temp	1	12.0	1.09	0.30	0.12	1	1.6	0.15	0.70	0.02					
Fe x temp	1	4.2	0.38	0.54	0.04	1	4.2	0.38	0.54	0.04					
Residuals	7	31.8				7	74.5								

**Supplement Table S18:** Results of the Scheirer-Ray-Hare-Test for flow-cytometry based group specific net growth rates of phytoplankton in bioassay W2. Df: degrees of freedom, Sum Sq: sum of squares, H: H-test statistic,  $\eta^2$ : eta-squared (effect size), Fe: iron addition, temp: temperature increase, Fe x temperature: interaction of iron addition and temperature increase.

Variable	Phyto 2					Phyto 3					Phyto 4				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	0.3	0.03	0.87	0.00	1	5.3	0.41	0.52	0.04	1	108.0	8.31	0.00	0.76
temp	1	12.0	0.92	0.34	0.08	1	12.0	0.92	0.34	0.08	1	8.3	0.64	0.42	0.06
Fe x temp	1	1.3	0.10	0.75	0.01	1	3.0	0.23	0.63	0.02	1	1.3	0.10	0.75	0.01
Residuals	8	129.3				8	122.7				8	25.3			
Variable	Phyto 6					Phyto 7					Phyto 8				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	56.3	4.33	0.04	0.39	1	48.0	3.69	0.05	0.34	1	108.0	8.31	0.00	0.76
temp	1	8.3	0.64	0.42	0.06	1	12.0	0.92	0.34	0.08	1	1.3	0.10	0.75	0.01
Fe x temp	1	0.3	0.03	0.87	0.00	1	8.3	0.64	0.42	0.06	1	0.3	0.03	0.87	0.00
Residuals	8	78.0				8	74.7				8	33.3			
Variable	Phyto 12					Phyto 13					Phyto 16				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	96.3	7.41	0.01	0.67	1	65.3	5.03	0.02	0.46	1	108.0	8.31	0.00	0.76
temp	1	16.3	1.26	0.26	0.11	1	1.3	0.10	0.75	0.01	1	16.3	1.26	0.26	0.11
Fe x temp	1	0.3	0.03	0.87	0.00	1	27.0	2.08	0.15	0.19	1	1.3	0.10	0.75	0.01
Residuals	8	30.0				8	49.3				8	17.3			
Variable	Phyto 17					Phyto 22					Phyto 23				
	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$	Df	Sum Sq	H	p-value	$\eta^2$
Fe	1	108.0	8.31	0.00	0.76	1	1.3	0.10	0.75	0.01	1	108.0	8.31	0.00	0.76
temp	1	5.3	0.41	0.52	0.04	1	12.0	0.92	0.34	0.08	1	3.0	0.23	0.63	0.02
Fe x temp	1	8.3	0.64	0.42	0.06	1	0.3	0.03	0.87	0.00	1	1.3	0.10	0.75	0.01
Residuals	8	21.3				8	129.3				8	30.7			
Variable	Phyto 24														
	Df	Sum Sq	H	p-value	$\eta^2$										
Fe	1	96.3	7.41	0.01	0.67										
temp	1	8.3	0.64	0.42	0.06										
Fe x temp	1	0.3	0.03	0.87	0.00										
Residuals	8	38.0													

**Supplement Table S19:** Statistical parameters of the generalized linear model (GLM) testing the single and interaction effect of iron and temperature increase and Fe-addition on total phytoplankton abundances across all bioassay experiments. Bioassay experiment and day number (excluding the start of the experiment) were added as explanatory factors without interaction (Model formula: total phytoplankton abundances ~ Fe treatment \* temperature treatment + bioassay name + day number, with a quasipoisson distribution and log-link). Bioassay A2 was part of the intercept. CI is the 95% confidence interval.

	Coefficient	Lower CI	Upper CI
Fe-addition	0.03	-0.05	0.12
Temperature increase	0.11	0.02	0.19
Interaction Fe-Temp.	0.12	0.01	0.24
Bioassay A1	0.84	0.76	0.93
Bioassay W1	-0.51	-0.63	-0.40
Bioassay W2	0.06	-0.04	0.16
Day number 2	0.25	0.10	0.39
Day number 3	0.51	0.37	0.65
Day number 4	0.69	0.55	0.83
Day number 5	0.88	0.70	1.06
Day number 6	1.16	1.04	1.28
Day number 8	1.42	1.26	1.57

**Supplement Table S20:** Total macronutrient drawdown over the time of incubation. Starting concentrations were averaged and subtracted with end concentrations to calculate total nutrient drawdown. C: control, F: iron-addition treatment, T: temperature treatment, TF: iron-temperature treatment. NOx: NO<sub>2</sub> + NO<sub>3</sub>.

Bioassay	Treatment	Replicate	NOx drawdown [ $\mu$ M]	PO <sub>4</sub> drawdown [ $\mu$ M]	Si drawdown [ $\mu$ M]
A1	C	A	8.03	0.85	5.42
A1	C	B	5.25	0.89	5.84
A1	C	C	6.38	0.98	6.66
A1	F	A	12.87	1.03	5.16
A1	F	B	11.84	1.07	6.06
A1	F	C	5.48	1.00	4.43
A1	T	A	13.43	1.10	9.62
A1	T	B	9.58	0.92	8.86
A1	T	C	9.71	0.95	8.82
A1	TF	A	10.67	1.20	8.96
A1	TF	B	13.66	1.16	7.77
A1	TF	C	13.85	1.16	7.73
A2	C	A	-7.35	0.13	1.65
A2	C	B	-1.40	0.16	1.40
A2	C	C	-3.41	0.17	1.43
A2	F	A	-3.84	0.20	1.53
A2	F	B	-3.69	0.13	1.19
A2	F	C	-1.18	0.18	1.45
A2	T	A	-1.43	0.20	1.82
A2	T	B	-1.59	0.21	1.51
A2	T	C	-3.22	0.21	2.57
A2	TF	A	-1.91	0.19	2.00
A2	TF	B	-13.79	0.17	1.58
A2	TF	C	-1.92	0.21	1.89
W1	C	B	4.62	0.42	11.10
W1	C	C	6.02	0.57	12.00
W1	F	A	13.15	1.01	15.06
W1	F	B	12.85	0.99	14.80
W1	F	C	13.16	1.00	14.73
W1	T	A	6.10	0.60	14.42
W1	T	B	6.04	0.56	13.59
W1	T	C	7.19	0.63	15.28
W1	TF	A	16.56	1.21	19.76
W1	TF	B	15.56	1.15	19.11
W1	TF	C	16.82	1.23	21.30
W2	C	A	5.39	0.42	5.90
W2	C	B	5.82	0.41	4.97
W2	C	C	7.70	0.46	5.51
W2	F	A	12.95	0.78	5.06
W2	F	B	14.75	0.87	5.52
W2	F	C	13.35	0.76	5.25
W2	T	A	5.93	0.46	7.89

W2	T	B	6.96	0.47	8.18
W2	T	C	9.42	0.63	10.28
W2	TF	A	18.93	1.14	10.74
W2	TF	B	19.92	1.20	10.96
W2	TF	C	19.14	1.16	11.33