

S1. Supplementary information about nitrogen fixation

S1.1. Estimating the potential for nitrogen fixation

Anvi'o v7.1 was used to estimate the metabolic potential for nitrogen fixation in euphotic layer samples where significant Ni isotope fractionation was detected (stations 8, 11 and 25), using the commands and parameters described in <https://anvio.org/blog/targeted-binning/>. This approach relies on anvi-estimate-metabolism to estimate the completeness of KEGG modules in contigs of individual metagenomic samples, working at operon-level. It allows to detect complete or partial nitrogen fixation operons instead of traditional single-gene *nifH* detection.

S1.2. Nitrogen fixation and Ni fractionation

Among the 13 omic samples corresponding to high $\delta^{60}\text{Ni}$ (11-15m, 11-30m, 8-15m, 8-40m, 25-15m, 25-30m, each with two size fractions except 25-15 m which has three), none showed a contig bearing more than 50 % of the genes from the M00175 KEGG module, corresponding to the catalytic part of the *nif* based nitrogen fixation. Nitrogen fixation potential could have been missed due to single assemblies not properly recovering contigs of nitrogen-fixing populations. Yet, similar metagenomic-based approaches have demonstrated their efficiency (Delmont et al. 2022), and recent data has shown evidence of limited nitrogen fixation in the Southern Ocean (Gu et al. 2025). Thus, the effect of nitrogen fixation on nickel fractionation in our samples should be negligible, if not absent.

References from S1

Delmont, T. O., J. J. Pierella Karlusich, I. Veseli, J. Fuessel, A. M. Eren, R. A. Foster, C. Bowler, P. Wincker, E. Pelletier: Heterotrophic bacterial diazotrophs are more abundant than their cyanobacterial counterparts in metagenomes covering most of the sunlit ocean, *The ISME Journal*, 16: 927-936, doi: 10.1038/s41396-021-01135-1, 2022.

Gu, S., Berthelot H., Lin Y., Tang W., Robidart J., Eren A. M., Ducklow H. W., and Cassar N.: Evidence of limited N_2 fixation in the Southern Ocean, *Communications Earth & Environment* 6: 264. doi:10.1038/s43247-025-02225-0, 2025

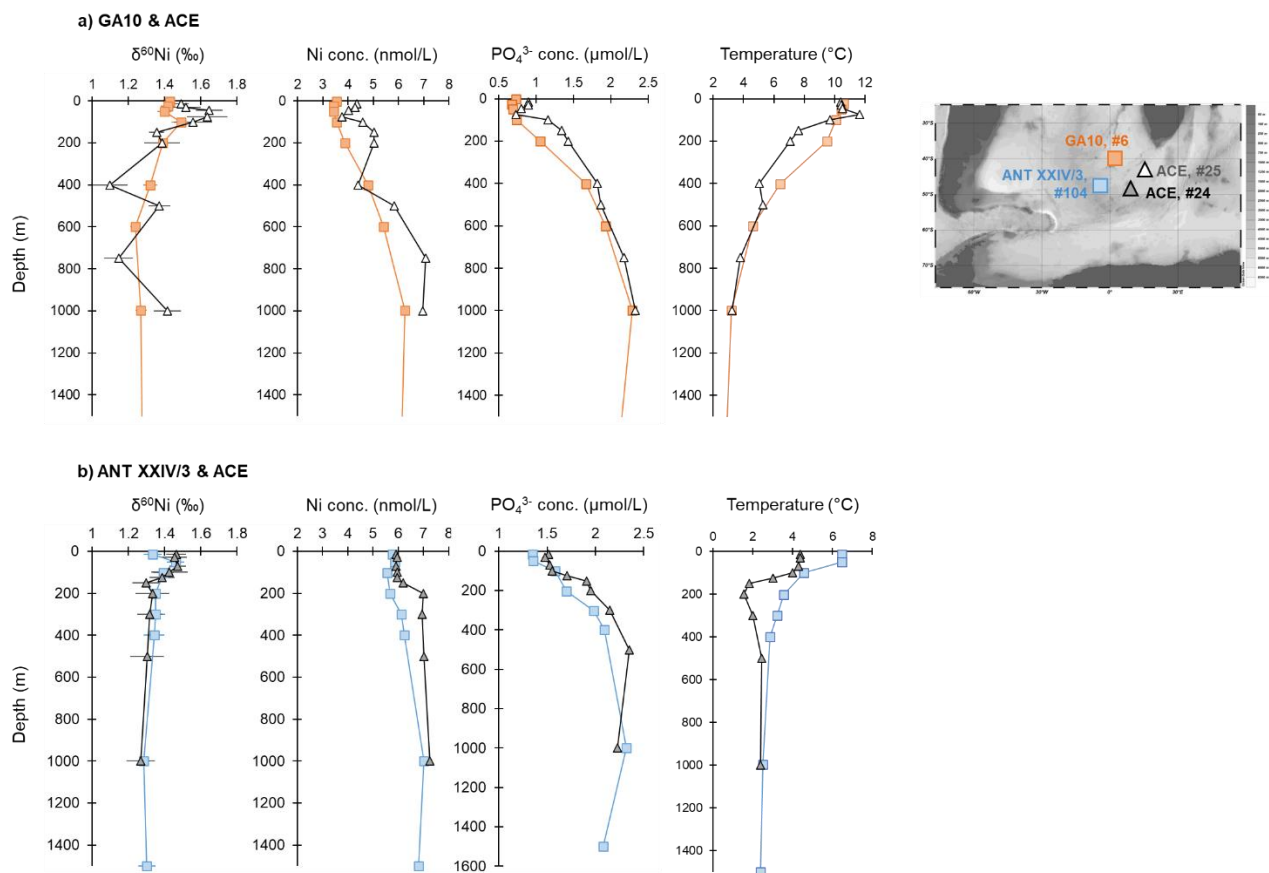


Figure S1: Comparison of depth profiles a) near the Sub-Tropical Front between ACE station #25 and GA10 station #6 (in orange; Archer et al., 2020), and b) near the Polar Front between ACE station #24 and ANT XXIV/3 station #104 (in blue; Cameron and Vance, 2014).

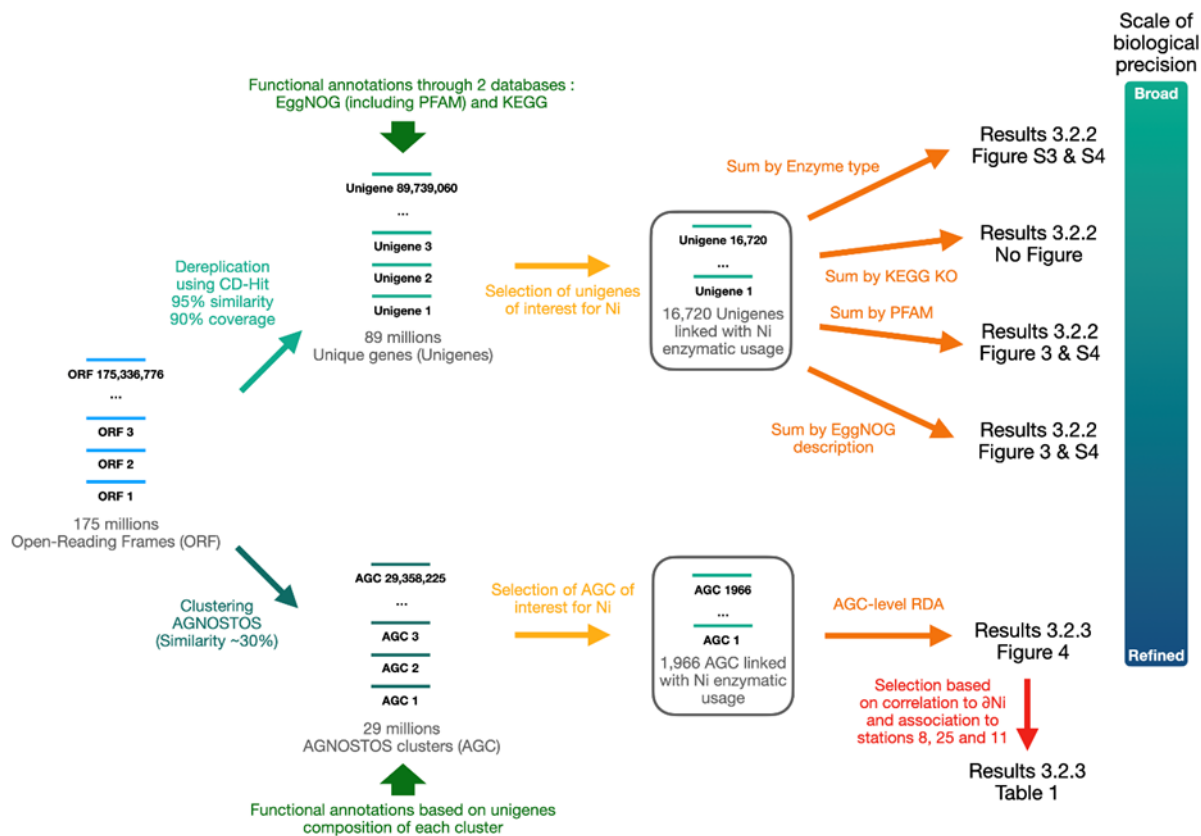
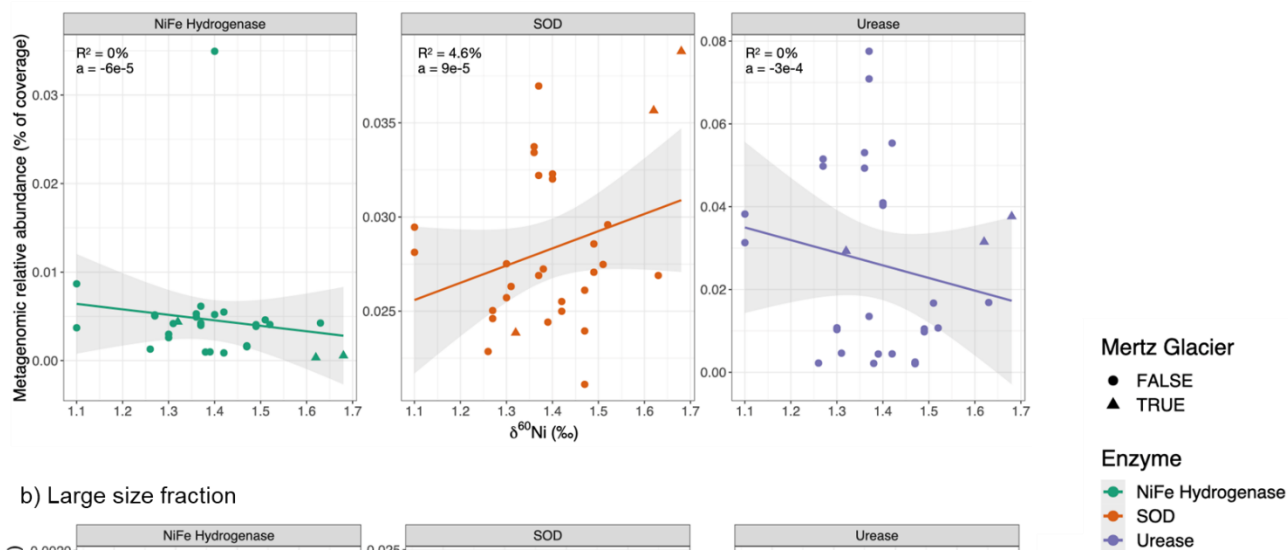


Figure S2: Schematic of the different methods applied to the metagenomic data from a broad biological scale (function-level investigation) to a refined biological scale (gene clusters-level investigation).

a) Small size fraction



b) Large size fraction

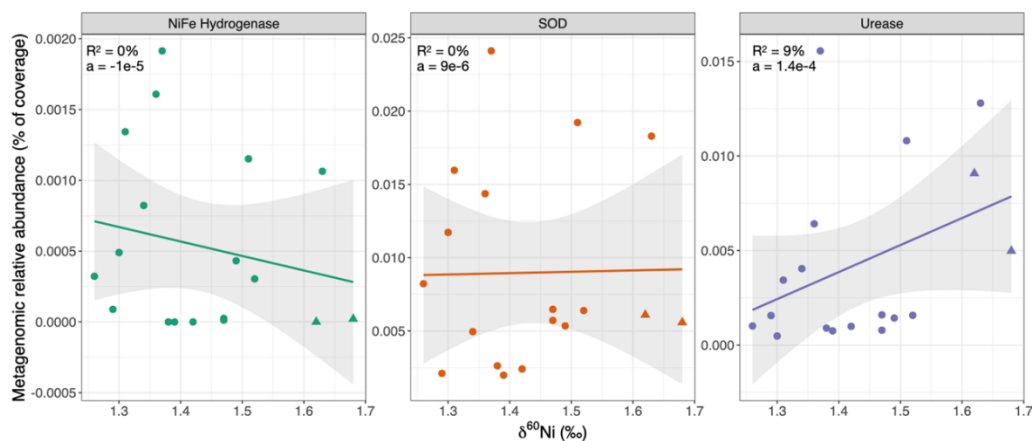


Figure S3: Comparison of Ni isotope composition ($\delta^{60}\text{Ni}$) and metagenomic relative abundance of broad functional groups: NiFe hydrogenase (in green, left plots), SOD (in orange, middle plots) and urease (in purple, right plots) in a) the small size fraction (0.2 – 3 μm and 0.2 – 40 μm ; top panels) and b) large size fraction (3 – 200 μm ; bottom panels). Samples from station 11, near the Mertz Glacier, are identified by triangle symbols. A regression line coloured by enzyme surrounded by a 95% confidence interval plotted in grey is represented for each plot.

a) Small size fraction

b) Large size fraction

c) Small size fraction

d) Large size fraction

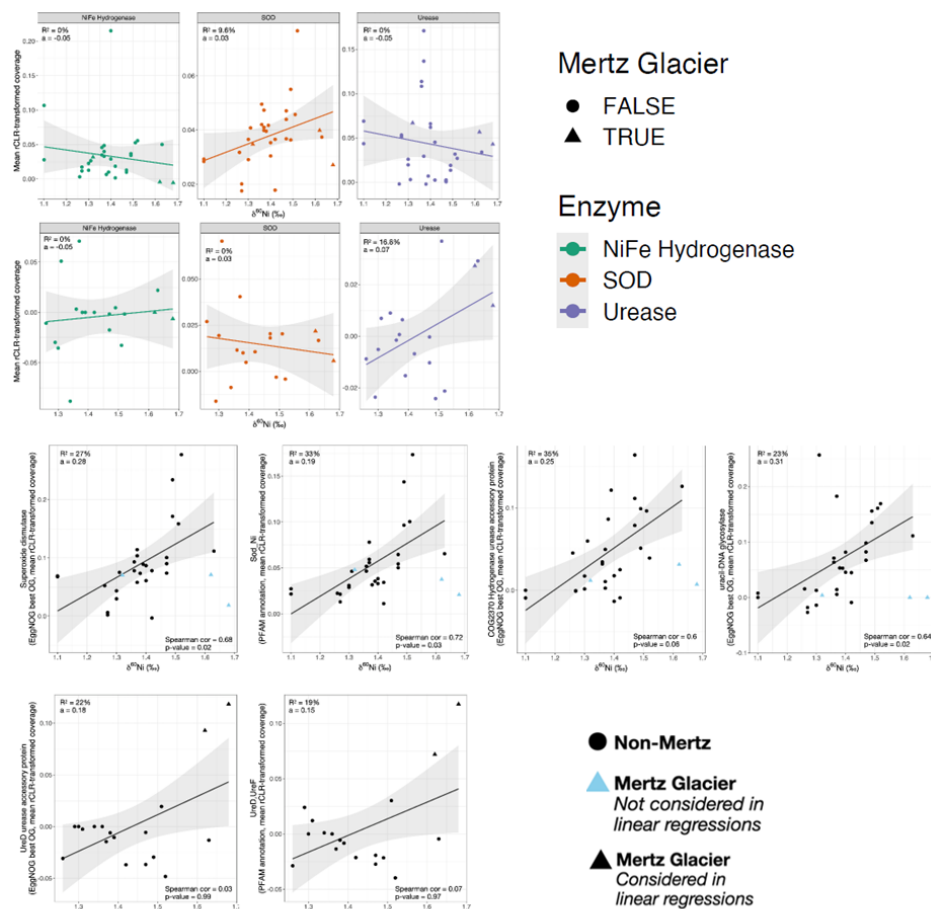


Figure S4: Comparison of Ni isotope composition ($\delta^{60}\text{Ni}$) and robust Centered-Log Ratio (r-CLR) transformed metagenomic abundance of broad functional groups: NiFe hydrogenase (in green, left plots), SOD (in orange, middle plots) and urease (in purple, right plots) in panels a (small size fraction) and b (large size fraction); EggNOG best OG descriptions or PFAM annotations in panels c (small size fraction) and d (large size fraction). A regression line surrounded by a 95% confidence interval plotted in grey is represented for each plot. Light blue symbols were excluded from the computation of the linear regression lines. Samples from station 11, near the Mertz Glacier, are identified by triangle symbols.

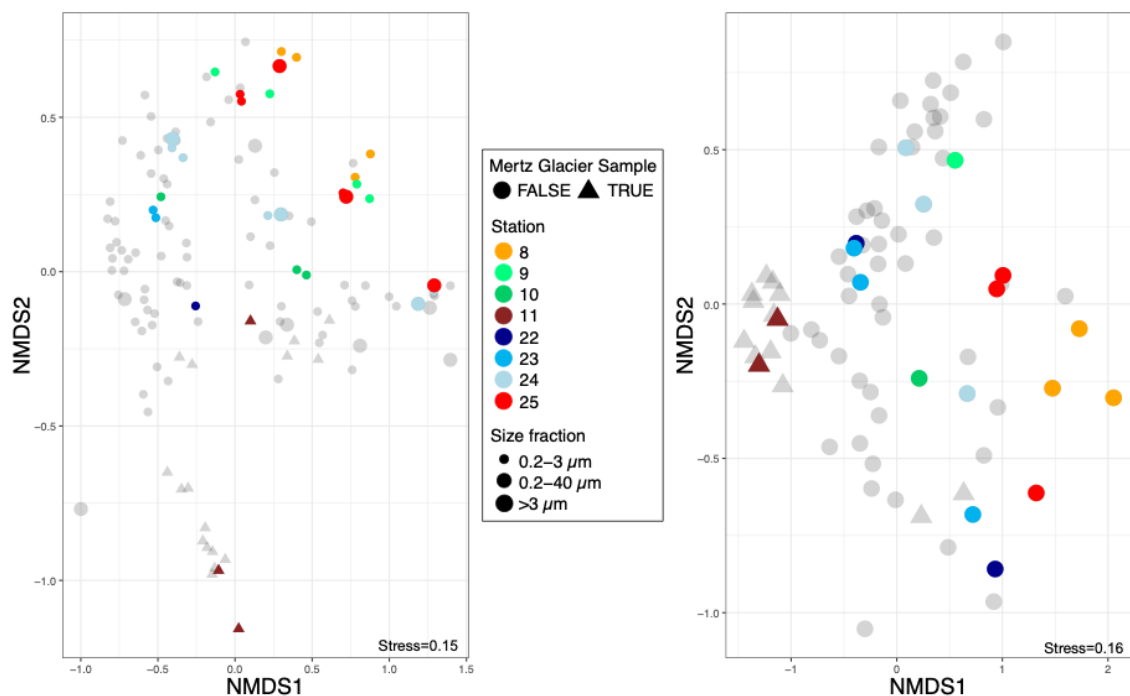
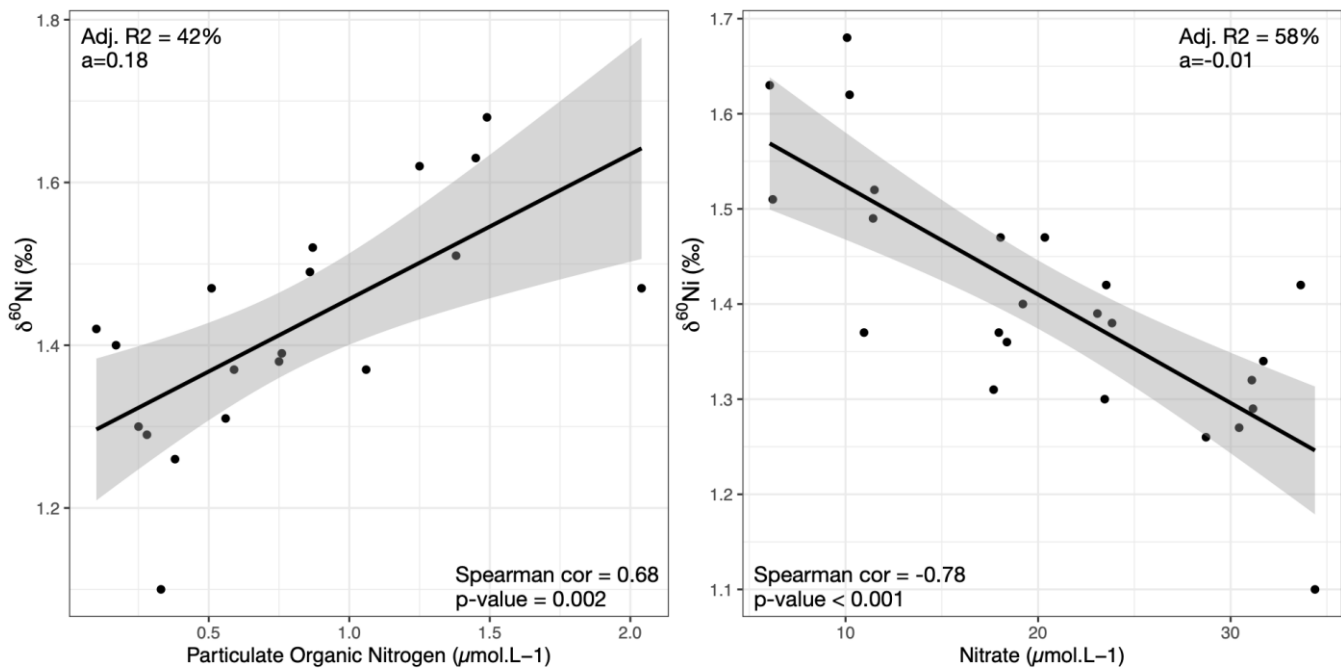


Figure S5: Differences in microbial communities across the 8 stations investigated. Code and data from Faure et al. (2026) were retrieved and used to reproduce the non-metric multidimensional scaling (NMDS) of the 218 ACE metagenomes, computed on the abundance matrix of AGNOSTOS gene clusters in the small (left panel) and large (right panel) size fractions. The 48 samples used in this study (*i.e.*, for which Ni concentrations and isotope compositions were available) are represented in colours, while the rest of the ACE samples are shown in grey. Colours indicate the station of sampling, the size of dots matches the size-fraction, and the shape distinguishes Mertz glacier samples (*i.e.*, station 11 represented by triangles) from the rest (represented by dots). Positions of the symbols reflect their composition in gene clusters.



65 **Figure S6: Correlation analysis of the relationship between Ni isotope composition ($\delta^{60}\text{Ni}$) and particulate organic nitrogen (PON; left panel) and nitrate (right panel) concentrations. Each dot represents a sample, spearman correlation coefficients and their corresponding p-values are shown at the bottom of each plot. A regression line surrounded by a 95% confidence interval plotted in grey is represented for each plot. The corresponding adjusted R^2 and coefficient are shown at the top of each graph.**

Station	Lat	Long	Depth	Potential density	Temperature	Salinity	Oxygen	Silicate	Nitrate	Phosphate	Ni	$\delta^{60}\text{Ni}$	2SD
#	°N	°E	m	kg/m ³	°C	psu	μmol/L	μmol/L	μmol/L	μmol/L	nmol/L	‰	‰
8	-46.39	150.39	15		12.49	35.60	245.10	0.45	6.27	0.53	2.97	1.63	0.08
8	-46.39	150.39	40	26.41	12.14	34.80	249.48	0.41	6.43	0.51	3.13	1.51	0.04
8	-46.39	150.39	60	26.43	12.06	34.80	246.55	0.48	6.58	0.55	3.35	1.39	0.10
8	-46.39	150.39	80	26.45	11.96	34.80	244.24	0.73	8.08	0.60	3.54	1.40	0.05
8	-46.39	150.39	125	26.55	11.44	34.81	243.30	1.97	10.98	0.72	3.76	1.45	0.11
8	-46.39	150.39	150	26.69	10.71	34.82	241.08	2.90	11.94	0.79	3.68	1.37	0.05
8	-46.39	150.39	200	26.78	10.19	34.81	240.41	3.33	12.16	0.78	3.57	1.39	0.08
8	-46.39	150.39	250	26.78	10.17	34.81	240.94	3.30	12.11	0.79	3.55	1.31	0.11
8	-46.39	150.39	300	26.78	10.12	34.80	241.58	3.32	12.42	0.77	3.66	1.16	0.07
8	-46.39	150.39	500	26.83	8.91	34.60	236.07	4.78	16.11	0.96	4.13	1.24	0.11
8	-46.39	150.39	750	26.88	8.29	34.53	237.44	5.31	16.84	1.08	4.26	1.39	0.09
9	-53.58	149.30	15	26.55	8.00	34.07	271.37	5.58	18.57	1.20	5.00	1.31	0.05
9	-53.58	149.30	30	26.55	7.99	34.07	270.60	5.34	18.66	1.24	4.94	1.36	0.06
9	-53.58	149.30	50	26.76	7.59	34.26	270.30	5.42	18.65	1.19	4.82	1.37	0.05
9	-53.58	149.30	80	26.84	6.97	34.26	264.55	5.22	17.90	1.16	4.77	1.43	0.07
9	-53.58	149.30	125	26.87	6.89	34.28	258.61	5.86	19.38	1.10	4.77	1.48	0.08
9	-53.58	149.30	150	26.89	6.84	34.29	252.38	6.56	20.53	1.16	4.81	1.40	0.07
9	-53.58	149.30	200	26.91	6.24	34.22	257.33	8.13	22.13	1.32	4.94	1.32	0.04
9	-53.58	149.30	250	26.94	5.98	34.21	251.86	9.41	22.97	1.37	5.09	1.19	0.08
9	-53.58	149.30	300	26.97	5.82	34.23	242.66	11.33	24.35	1.36	5.54	1.20	0.06
9	-53.58	149.30	500	27.11	4.82	34.25	213.17	21.72	29.22	1.76	5.62	1.28	0.09
9	-53.58	149.30	750	27.29	3.60	34.31	191.51	40.70	33.51	2.01	6.34	1.15	0.06
9	-53.58	149.30	1000	27.44	2.97	34.43	167.45	60.14	34.80	2.10	6.80	1.09	0.08
10	-59.61	148.64	30	27.04	2.90	33.93	309.21	22.86	25.88	1.66			
10	-59.61	148.64	50	27.20	1.45	33.98	317.80	33.95	27.88	1.91	6.52	1.37	0.08
10	-59.61	148.64	80	27.38	-0.49	34.07	323.40	39.11	28.54	1.98	6.50	1.26	0.06
10	-59.61	148.64	100	27.41	-0.62	34.09	318.07	42.35	29.04	1.99	6.59	1.24	0.10
10	-59.61	148.64	125	27.48	0.22	34.23	267.85	53.62	32.39	2.15	6.68	1.30	0.06
10	-59.61	148.64	150	27.53	1.33	34.38	199.81	64.98	34.62	2.28	6.69	1.10	0.04
10	-59.61	148.64	200	27.58	1.79	34.48	175.61	71.28	34.99	2.27	6.88	1.20	0.09
10	-59.61	148.64	250	27.61	2.12	34.55	161.72	74.93	34.78	2.21	6.82	1.23	0.07
10	-59.61	148.64	500	27.71	2.11	34.67	164.68	80.34	32.62	2.07	6.79	1.40	0.08
10	-59.61	148.64	750	27.76	1.98	34.72	172.08	84.19	30.95	2.02	6.72	1.33	0.08
11	-67.10	144.92	15	26.16	-0.99	32.53	372.04	38.12	10.02	0.62	4.95	1.68	0.04
11	-67.10	144.92	30	26.17	-1.05	32.54	374.27	38.03	10.51	0.70	5.05	1.62	0.04
11	-67.10	144.92	50	26.23	-1.22	32.61	376.15	40.81	11.78	0.78	5.05	1.65	0.04
11	-67.10	144.92	80	27.38	-0.89	34.04	359.38	60.80	20.68	1.42	7.15	1.43	0.05
11	-67.10	144.92	100	27.61	-1.43	34.30	313.04	69.52	25.49	1.73	7.08	1.36	0.05
11	-67.10	144.92	200	27.80	-1.99	34.51	286.81	75.99	30.69	1.76	6.86	1.32	0.03
11	-67.10	144.92	250	27.80	-2.00	34.51	286.42	77.39	30.78	1.88	7.74	1.32	0.04
11	-67.10	144.92	300	27.80	-1.99	34.52	285.73	78.02	30.83	1.83	8.03	1.31	0.06
11	-67.10	144.92	400	27.82	-2.01	34.54	285.56	77.94	30.77	1.81	6.86	1.30	0.04
11	-67.10	144.92	475	27.84	-2.08	34.56	286.70	77.55	30.75	1.92	7.93	1.36	0.08
22	-56.99	-27.95	10	27.00	1.66	33.75	312.80	49.58	23.58	1.63	5.86	1.32	0.06
22	-56.99	-27.95	30	27.00	1.67	33.75	312.02	49.65	23.58	1.62	6.39	1.38	0.04
22	-56.99	-27.95	60	27.33	0.07	34.04	303.87	55.12	24.73	1.73	7.35	1.36	0.05
22	-56.99	-27.95	85	27.49	-0.85	34.18	284.17	72.09	28.50	2.01	6.33	1.36	0.06
22	-56.99	-27.95	100	27.53	-0.70	34.24	267.05	76.42	30.30	2.18	7.61	1.36	0.05
22	-56.99	-27.95	150	27.66	0.14	34.45	223.55	86.35	32.17	2.21	6.09	1.34	0.07
22	-56.99	-27.95	200	27.73	0.56	34.56	202.47	91.69	32.20	2.22	6.23	1.20	0.09
22	-56.99	-27.95	300	27.78	0.80	34.64	191.58	97.63	32.10	2.21	6.10	1.34	0.10
22	-56.99	-27.95	500	27.79	0.68	34.65	190.49	104.04	32.18	2.20	8.29	1.28	0.07
22	-56.99	-27.95	800	27.81	0.55	34.66	190.11	111.28	32.32	2.28	6.38	1.25	0.08
22	-56.99	-27.95	1000	27.82	0.48	34.67	190.73	113.40	32.59	2.27	6.28	1.33	0.09
23	-58.67	-14.00	15	26.92	1.85	33.67	314.40	49.41	23.08	1.62	6.39	1.39	0.05
23	-58.67	-14.00	50	26.94	1.77	33.69	313.48	52.91	23.54	1.66	6.44	1.42	0.06
23	-58.67	-14.00	60	26.99	1.39	33.71	314.36	57.50	24.13	1.72	6.47	1.38	0.09
23	-58.67	-14.00	100	27.39	-0.83	34.06	312.27	64.78	27.18	1.95	6.62	1.37	0.06
23	-58.67	-14.00	125	27.49	-0.82	34.18	280.40	73.72	30.04	1.95	7.07	1.31	0.06
23	-58.67	-14.00	150	27.57	-0.32	34.31	252.61	80.09	31.06	2.18	6.93	1.29	0.06
23	-58.67	-14.00	250	27.73	0.79	34.58	193.77	88.28	31.78	2.17	7.36	1.33	0.10
23	-58.67	-14.00	500	27.80	0.72	34.66	188.30	104.45	31.87	2.25	7.33	1.27	0.06
23	-58.67	-14.00	750	27.82	0.59	34.67	185.87	111.51	31.88	2.23	7.31	1.38	0.08
23	-58.67	-14.00	1000	27.82	0.49	34.67	187.62	114.67	32.22	2.29	7.40	1.32	0.08
24	-49.00	9.00	15	26.79	4.39	33.79	292.61	3.33	20.33	1.52	5.92	1.47	0.05
24	-49.00	9.00	30	26.79	4.38	33.80	293.11	3.35	20.41	1.48	5.95	1.46	0.07
24	-49.00	9.00	70	26.81	4.28	33.80	292.17	3.45	20.27	1.53	5.92	1.47	0.05
24	-49.00	9.00	100	26.85	4.00	33.82	290.96	4.17	20.43	1.56	5.95	1.43	0.10
24	-49.00	9.00	125	26.98	3.01	33.87	291.48	11.47	22.34	1.71	5.97	1.39	0.07
24	-49.00	9.00	150	27.15	1.81	33.95	286.76	20.79	25.91	1.91	6.21	1.30	0.08
24	-49.00	9.00	200	27.21	1.56	34.01	277.80	27.00	28.06	1.95	7.01	1.33	0.09
24	-49.00	9.00	300	27.30	1.99	34.16	228.50	36.67	30.53	2.15	6.96	1.32	0.07
24	-49.00	9.00	500	27.45	2.45	34.38	175.12	58.56	33.54	2.35	7.03	1.30	0.09
24	-49.00	9.00	1000	27.67	2.38	34.65	161.78	74.70	31.81	2.23	7.25	1.27	0.08
25	-43.98	14.07	15	26.37	10.42	34.34	256.34	1.14	11.43	0.90	4.34	1.49	0.04
25	-43.98	14.07	30	26.37	10.43	34.34	255.68	1.12	11.50	0.90	4.27	1.52	0.08
25	-43.98	14.07	45	26.37	10.52	34.36	255.70	1.25	9.82	0.81	4.03	1.65	0.07
25	-43.98	14.07	75	26.40	11.68	34.67	244.19	1.37	8.65	0.74	3.75	1.64	0.11
25	-43.98	14.07	100	26.50	9.71	34.35	253.28	2.18	14.93	1.16	4.59	1.56	0.10
25	-43.98	14.07	150	26.74	7.62	34.24	253.40	5.65	18.77	1.34	5.04	1.36	0.04
25	-43.98	14.07	200	26.86	7.08	34.30	245.31	7.17	20.37	1.43	5.02	1.39	0.10
25	-43.98	14.07	400	27.03	5.04	34.19	228.42	12.21	26.39	1.82	4.39	1.10	0.10
25	-43.98	14.07	500	27.10	5.28	34.31	203.15	21.21	27.59	1.87	5.84	1.37	0.06
25	-43.98	14.07	750	27.26	3.79	34.30	189.86	34.64	30.94	2.18	7.08	1.15	0.08
25	-43.98	14.07	1000	27.40	3.25	34.41	170.12	51.53	33.14	2.33	6.95	1.42	0.08

Table S1: Dissolved $\delta^{60}\text{Ni}$, Ni concentrations, silicate, nitrate, phosphate, potential density (σ_θ), temperature and salinity for 8 stations of the ACE cruise.

Table S2: Functional and taxonomic annotations of all AGNOSTOS gene clusters (AGC) emerging from the redundancy analyses as particularly linked to $\delta^{60}\text{Ni}$ (shown in yellow in Figure 4), station 11 (shown in red in Figure 4) and stations 8 and 25 (shown in orange in Figure 4), for the small (0.2 - 3 μm and 0.2 - 40 μm) and large (3 - 200 μm) size fractions.