Influence of Ni-related enzymes on the Ni cycle in the Southern Ocean: insights from isotopes and metagenomics

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Supplementary information about nitrogen fixation

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.

2) Nitrogen fixation and Ni fractionation

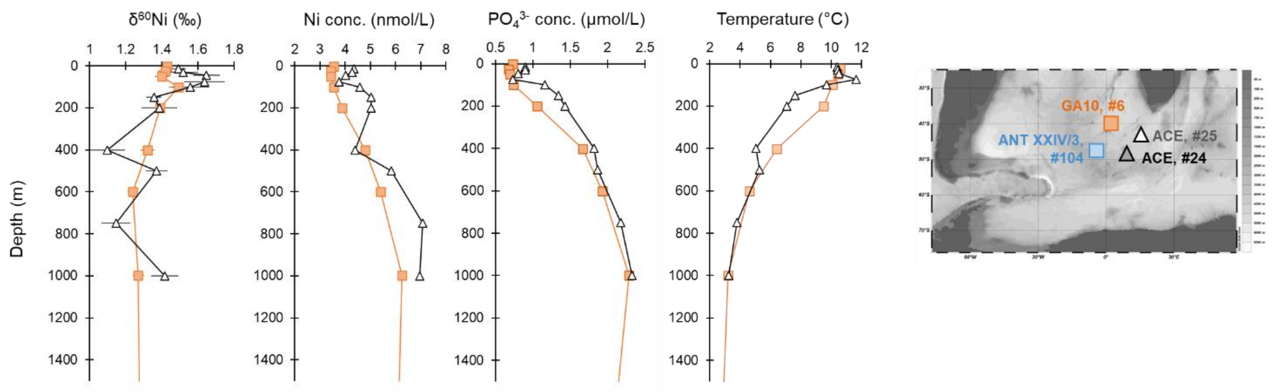
Among the 13 omic samples corresponding to high δ^{60}Ni (11-15m, 11-30m, 8-15m, 8-40m, 25-15m, 25-30m, each with two size fractions except 25-15 m which has three), none showed a contig bearing more than 50 % of the genes from the M00175 KEGG module, corresponding to the catalytic part of the nif based 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 Supplementary information

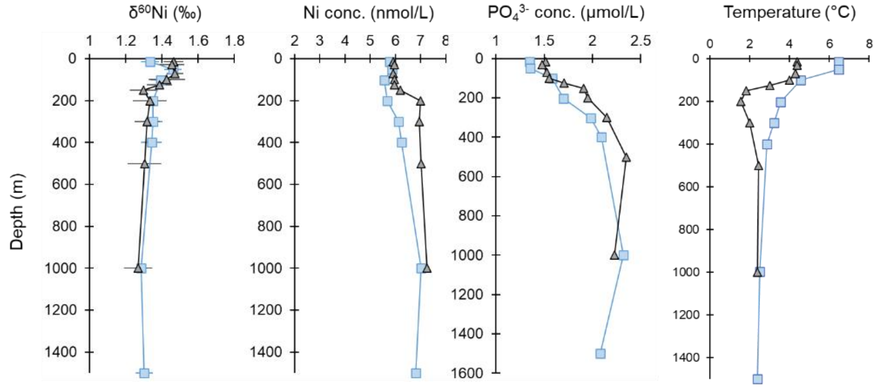
Delmont, T. O., J. J. Pierella Karlusich, I. Veseli, J. Fuessel, A. M. Eren, R. A. Foster, C. Bowler, P. Wincker, E. Pelletier: Heterotrophic bacterial diazotrophs are more abundant than their cyanobacterial counterparts in metagenomes covering most of the sunlit ocean, The ISME Journal, 16: 927-936,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

a) GA10 & ACE



b) ANT XXIV/3 & ACE



<u>Figure S1:</u> 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).

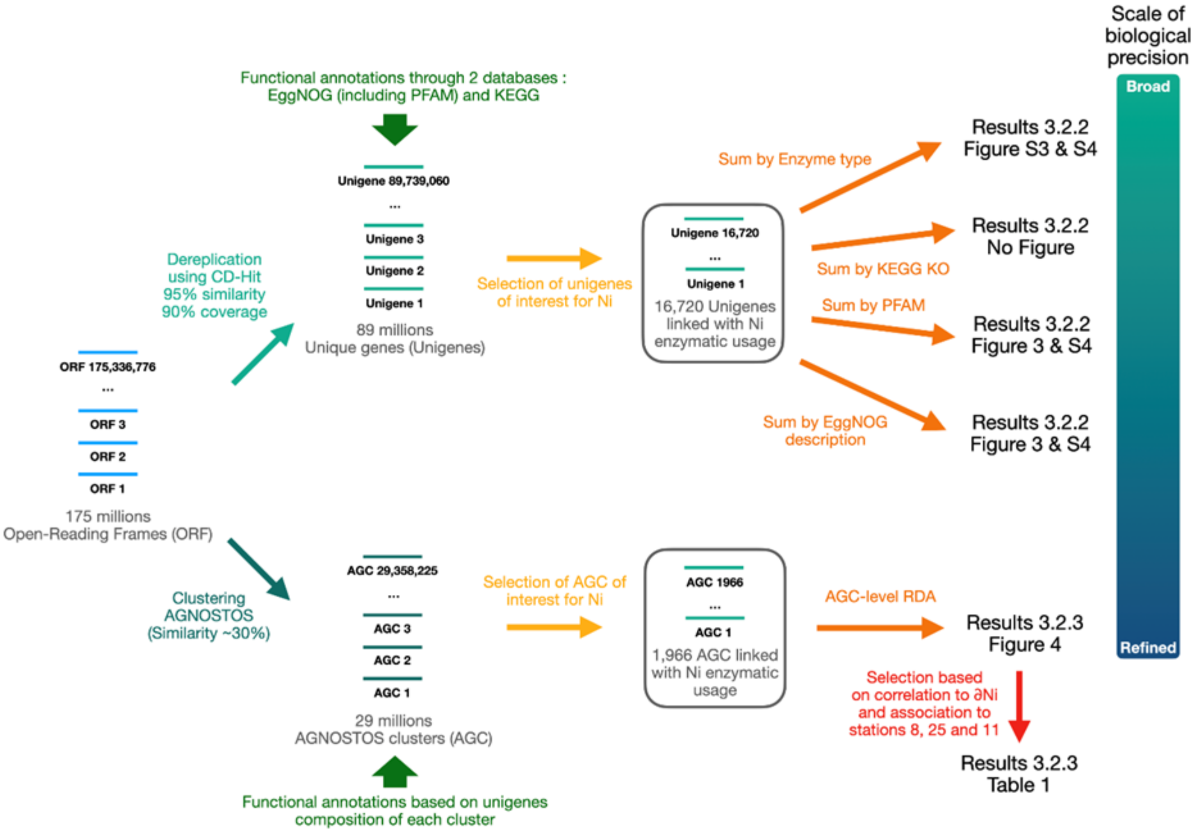
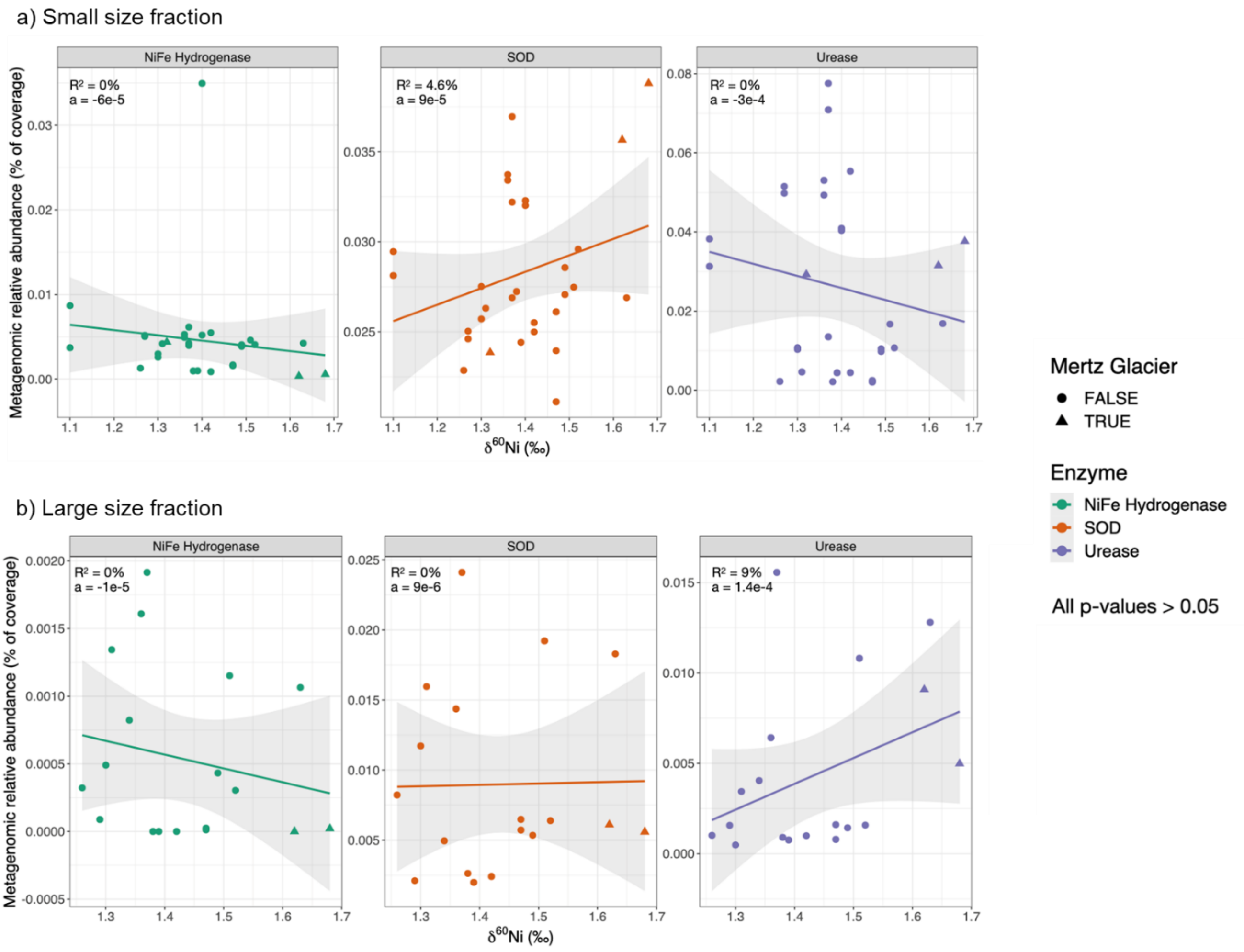


Figure S2: Schematic of the different methods applied to the metagenomic data from a broad biological scale (function-level investigation) to a refined biological scale (gene clusters-level investigation).



<u>Figure S3</u>: Comparison of Ni isotope composition (δ^{60}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.

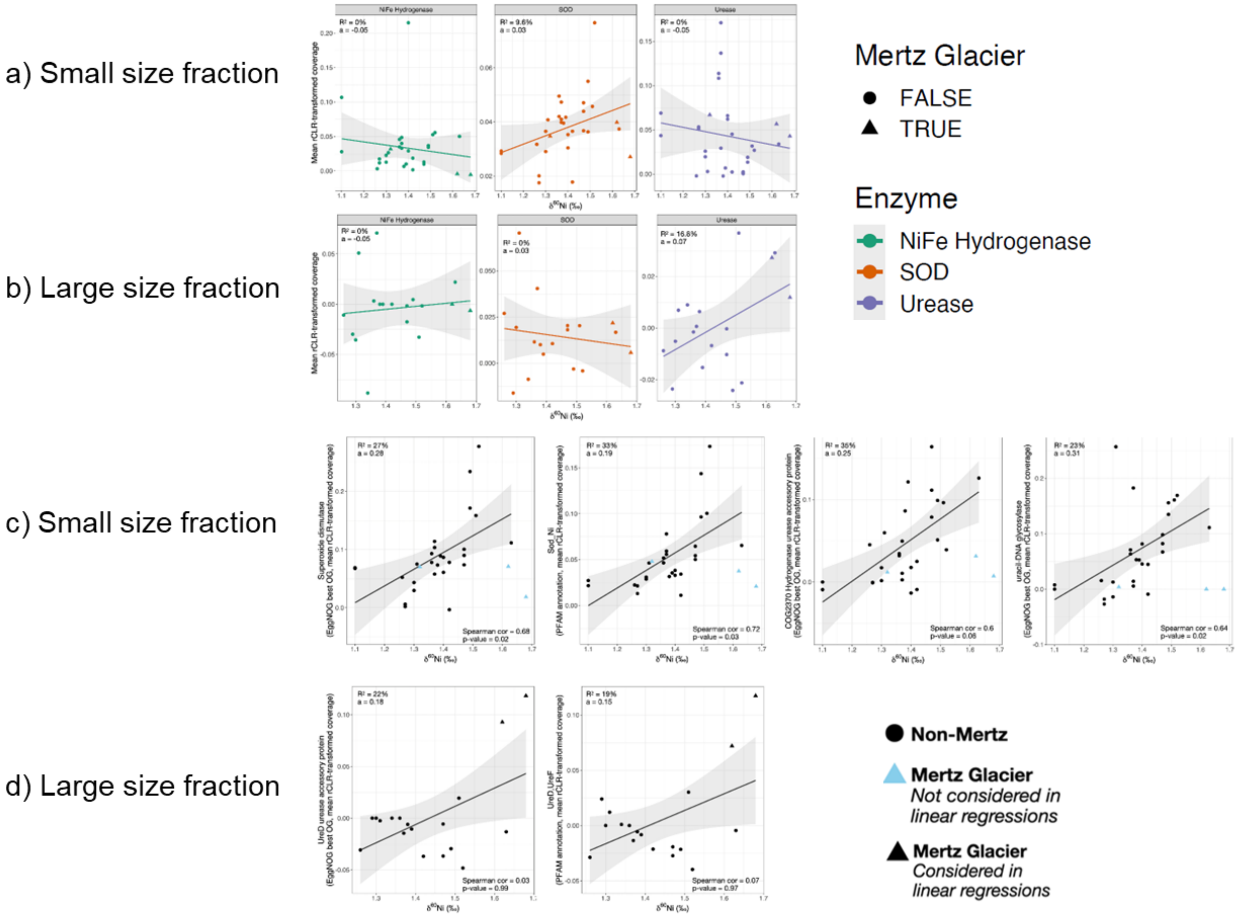


Figure S4: Comparison of Ni isotope composition (δ^{60}Ni) and robust Centered-Log Ratio (r-CLR) transformed metagenomic abundance of broad functional groups: NiFe hydrogenase (in green, left plots), SOD (in orange, middle plots) and urease (in purple, right plots) in panels a (small size fraction) and 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.

Station	Lat	Long	Depth	Potential density	Temperature	Salinity	Oxygen	Silicate	Nitrate	Phosphate	Ni	δ^{60}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	39.03	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 δ^{60}Ni, Ni concentrations, silicate, nitrate, phosphate, potential density (σ_{Θ}), temperature and salinity for 8 stations of the ACE cruise.

	Size fraction	AGNOSTOS genecluster	Size	EggNOG best OG description	PFAM	Highest Taxonomy (UniRef90)	Remarks on UniRef Taxonomy	Taxonomy (GTDB, no eukaryotes)
δ^{60}Ni	small	AGC_14548656	94	Required for maturation of urease via the functional incorporation of the urease nickel metallocenter	UreF	Undefined	Among annotated sequences, Roseobacteraceae and Rhodobacteraceae from Octadecabacter genus dominate	93 of 94 ORFs are annotated to Rhodobacteraceae contigs. Dominant genus is Octadecabacter (68 ORFs).
	small	AGC_23057241	72	Required for maturation of urease via the functional incorporation of the urease nickel metallocenter	UreD	Rhodobacteraceae		Mainly Rhodobacteraceae (38 ORFs), also Halieaceae, UBA2165 and other families with <3 ORFs. Main genera are Ascidiaceihabitans, Octadecabacter, Luminiphilus and Planktomarina.
	both	AGC_14687886	154	Involved in urease metallocenter assembly. Binds nickel. Probably functions as a nickel donor during metallocenter assembly	UreE_C,UreE_N	Undefined	Among annotated sequences, Roseobacteraceae and Rhodobacteraceae dominates; Loktanella / Sulfitobacter / Thalassobium are the most found genus	151 of 154 ORFs are annotated to Rhodobacteraceae contigs. Dominant genera are Yoonia (40 ORFs), Ruegeria (32 ORFs) Ascidiaceihabitans (23 ORFs) and Parasedimentitalea (13 ORFs).
	large	AGC_24852953	991	Uracil-DNA glycosylase	UDG	Bacteria	Only 39 Nickel-related ORFs, four distinct families+ majority of unknowns	Among the 39 Nickel-related ORFs, 30 are Alphaproteobacteria and 8 Acidimicrobiia. 20 of the ORFs match to an Arctic Ocean MAG (WLW201 sp905182615), 8 to the psychrophilic Qipengyuania citrea.
Station 11	small	AGC_17036882	149	Uracil-DNA glycosylase	UDG	Undefined	Among annotated sequences, unknown Nitrospinae and Rhodobacterales dominate	Nitrospinales (UBA8687 genus) and Rhodobacterales (Yoonia genus)
	small	AGC_14355128	444	Superoxide dismutase	Sod_Ni	Flavobacteriaceae; Polaribacter		Flavobacteriaceae (Winogradskyella, Maribacter, Psychroflexus, Paraglaciecola and Polaribacter genera)
	small	AGC_20916161	279	Superoxide dismutase	Sod_Ni	Undefined	Among annotated sequences, Porticoccaceae dominate (SAR92 family)	Porticoccaceae (HTCC2207 genus)
	small	AGC_1819471	120	Excises uracil residues from the DNA which can arise as a result of misincorporation of dUMP residues by DNA polymerase or due to deamination of cytosine	UDG	Flavobacteriaceae		Flavobacteriaceae (SGZD1 genus)
	small	AGC_22886549	101	Destroys radicals which are normally produced within the cells and which are toxic to biological systems	Sod_Fe_C,Sod_Fe_N	Alteromonadaceae; Glaciecola nitratireducens		6 families with at least 5 representent, Alteromonadaceae and GCA-001735895 are the most represented
	small	AGC_3062244	12	Pfam Secreted repeat of unknown function	CHRD	Flavobacteriaceae; Polaribacter / Psychroflexus		Flavobacteriaceae (Polaribacter and Urechidicola genera)
	small	AGC_5999524	32	Destroys radicals which are normally produced within the cells and which are toxic to biological systems	Sod_Fe_C,Sod_Fe_N	Flavobacteriales		Flavobacteriales (multiple genera including UWMA-0277, TMED14, UBA4465)
	small	AGC_10331396	22	Pfam Secreted repeat of unknown function	CHRD	Flavobacteriaceae; Psychroflexus gondwanensis		Flavobacteriaceae (7 genera including Polaribacter, Nonlabens, Cellulophaga and psychroflexus
	small	AGC_19486454	70	Destroys radicals which are normally produced within the cells and which are toxic to biological systems	Sod_Cu	Flavobacteriaceae		Flavobacteriaceae (5 genera including GCA-002733185 and Patiriisocius)
	small	AGC_28603842	14	Destroys radicals which are normally produced within the cells and which are toxic to biological systems	Sod_Fe_C,Sod_Fe_N	Flavobacteriaceae; Polaribacter		Flavobacteriaceae (Polaribacter genus)
	small	AGC_18521265	40	Destroys radicals which are normally produced within the cells and which are toxic to biological systems	Sod_Cu	Flavobacteriaceae; Polaribacter		Flavobacteriaceae (Polaribacter genus)
	small	AGC_10694464	15	Involved in urease metallocenter assembly. Binds nickel. Probably functions as a nickel donor during metallocenter assembly	UreE_C,UreE_N	Undefined	Among annotated sequences, Rhodobacteraceae dominate	Rhodobacteraceae (mainly Octadecabacter genus, also Ascidiaceihabitans, Parasedimentitalea and Planktomarina)
	small	AGC_14931481	42	Destroys radicals which are normally produced within the cells and which are toxic to biological systems	Sod_Fe_C,Sod_Fe_N	Undefined	Among annotated sequences, unknown Alteromonadales dominate	Mainly Pseudomonadaceae (Halopseudomonas genus) and Cellvibrionaceae (CANLBN01 and Gilvimarinus genera)
	large	AGC_2646602	35	Undefined	Undefined	Undefined	Contains only one identified nickel ORF : UreD urease accessory protein from Fragilariopsis cylindrus	Not applied
	large	AGC_2848081	33	Undefined	Undefined	Undefined	Contains only one indentified nickel ORF : UreD urease accessory protein from Timspurckia oligopyrenoides	Not applied
	large	AGC_2144826	91	UreD urease accessory protein	UreD,UreF	Bacillariaceae; Fragilariopsis cylindrus		Not applied
	large	AGC_5722614	98	oxidoreductase activity, acting on superoxide radicals as acceptor	GlcNAc,Sod_Cu	unknown family; Labyrinthulid		Not applied
	large	AGC_29276704	43	Belongs to the metallo-dependent hydrolases superfamily. Urease alpha subunit family	Amidohydro_1,Urease_alpha	Prymnesiaceae; Haptolina ericina		Not applied
	large	AGC_12915342	38	CobW/HypB/UreG, nucleotide-binding domain	cobW	Naviculaceae; Craspedostauros australis		Not applied
	large	AGC_6758156	7	Urease alpha-subunit, N-terminal domain	Amidohydro_1,Urease_alpha,Urease_beta, Urease_gamma	Bacillariaceae; Fragilariopsis cylindrus		Not applied
Stations 8 and 25	small	AGC_23334844	54	Facilitates the functional incorporation of the urease nickel metallocenter. This process requires GTP hydrolysis, probably effectuated by UreG	cobW	Rhodobacterales	Diverse families, Rhodobacteraceae, Roseobacteraceae, Rhodospirillaceae,...	Mainly Rhodobacteraceae, various genera (Yoonia, TMED109, Octadecabacter, Planktomarina,...)
	small	AGC_14840044	852	cellular macromolecule catabolic process	ubiquitin	Bacillariophyta	Contains 5 diatom SOD_Ni ORFs	Not applied
	small	AGC_13989208	275	Destroys radicals which are normally produced within the cells and which are toxic to biological systems	Sod_Fe_C,Sod_Fe_N	Rhodobacteraceae; Planktomarina		Mainly Rhodobacteraceae (113 ORFs, multiple genera incl. Planktomarina, AG-337-I02 and Yoonia) and Pelagibacteraceae (55 ORFs, Pelagibacter genus)
	small	AGC_11093182	20	Destroys radicals which are normally produced within the cells and which are toxic to biological systems	Sod_Cu	Bathycoccaceae; Bathycoccus prasinos		Not applied
	small	AGC_2164110	169	Superoxide dismutase	Sod_Ni	Undefined	Mainly Gammaproteobacteria, diverse families, Piscirickettsiaceae, Alteromonadaceae, Porticoccaceae,...	Mainly Gammaproteobacteria, 16 different families, Methylophagaceae (synonym of Piscirickettsiaceae), Methylophilaceae, Nitrincolaceae,...
	small	AGC_17472745	165	PFAM Nickel-dependent hydrogenase, large subunit	NiFeSe_Hases	Undefined	Among the few annotated sequences, Flavobacteriaceae dominate.	Mainly Cyclobacteriaceae (69 ORF, all anotated to UBA4465 sp943788235, a MAG from the North Sea) and Flavobacteriaceae (38 ORFs, mainly MS024-2A and Maribacter genera).
	small	AGC_18601609	31	Destroys radicals which are normally produced within the cells and which are toxic to biological systems	Sod_Fe_C,Sod_Fe_N	Prasinoderma singulare		Not applied
	small	AGC_24807388	114	urease activity	Amidohydro_1,DUF599,Fungal_trans,HMG_box,Urease_alpha,Urease_beta,Urease_gamma	Undefined	Eukaryotic	Not applied
	small	AGC_18108257	29	Copper chaperone for superoxide dismutase	HMA,Sod_Cu	Bathycoccaceae; Bathycoccus prasinos		Not applied
	small	AGC_8142656	22	Involved in urease metallocenter assembly. Binds nickel. Probably functions as a nickel donor during metallocenter assembly	UreE_C,UreE_N	Synechococcaceae; Synechococcus		Cyanobiaceae (Parasynechococcus genus)
	small	AGC_25996182	57	urease accessory protein	UreD,UreF	Undefined	Among the few annotated sequences, Chloropicaceae and Bathycoccaceae dominate	Not applied
	small	AGC_26196954	31	Nickel-containing superoxide dismutase	Sod_Ni	Phaeocystaceae; Phaeocystis antarctica		Not applied
	small	AGC_29397748	240	Nickel-containing superoxide dismutase	Sod_Ni	Undefined	Diverse families, Planctomycetaceae, Opitutaceae,...	Diverse families from two main orders : Pirellulales (syn. Planctomycetales) and Opitutales. Main genera are UBA1268 (74 ORFs) and MB11C04 (53 ORFs)
	small	AGC_4137832	45	Pfam Secreted repeat of unknown function	CHRD	Planctomycetaceae		All ORFs annotated to UBA1268 genus (UBA1268 family in GTDB, Planctomycetaceae in NCBI)
	small	AGC_28830756	25	Belongs to the peptidase S8 family / calcium- and calmodulin-responsive adenylate cyclase activity / self proteolysis / domain, Protein / domain protein / -	S_nucleotid_C,ASH,Autotransporter [...], VCBS, VPEP, VWA, VWD, Wxl, fn3	Planctomycetaceae	Only 3 nickel-related ORF	All nickel-related ORFs annotated to UBA1268 genus
	small	AGC_3526244	21	Pfam Secreted repeat of unknown function	CHRD	Planctomycetaceae		19 of the 20 nickel-related ORFs annotated to UBA1268 genus
	small	AGC_20290509	18	Belongs to the peptidase S8 family / calcium- and calmodulin-responsive adenylate cyclase activity / self proteolysis / domain, Protein / domain protein / -	S_nucleotid_C,ASH,Autotransporter [...], VCBS, VPEP, VWA, VWD, Wxl, fn3	Planctomycetaceae	Only 1 nickel-related ORF	Nickel-related ORF is annotated to UBA1268 genus
	small	AGC_27773751	20	Pfam Secreted repeat of unknown function	CHRD	Planctomycetaceae		14 of the 15 nickel-related ORFs annotated to UBA1268 genus
	small	AGC_5500562	40	Pfam Secreted repeat of unknown function	Fasciclin,Lipoprotein_15	Rhodobiaceae	Only 1 nickel-related ORF	MED-G09 genus
	small	AGC_19008763	57	uracil-DNA glycosylase	DUF4130,UDG	Rhodobiaceae	Only 1 nickel-related ORF	MED-G09 genus
	small	AGC_21493453	71	uracil-DNA glycosylase	UDG	Archaea	Only 1 nickel-related ORF	Archaea of the MGII order
	small	AGC_11234517	38	Pfam Secreted repeat of unknown function	CHRD	Planctomycetaceae		29 of the 30 nickel-related ORFs annotated to UBA1268 genus
	both	AGC_13479537	67	Belongs to the urease gamma subunit family	Urease_beta,Urease_gamma	Undefined	Blend of Proteobacteria, Thaumarchaeota and others	19 ORFs anotated as Archaea from the Nitrosopumilaceae family, 39 ORFs annotated as various proteobacteria (incl. Alteromonadaceae and Rhodobacteraceae).
	both	AGC_7792447	2463	Destroys radicals which are normally produced within the cells and which are toxic to biological systems	Sod_Fe_C,Sod_Fe_N	Undefined	Among annotated sequences, Rhodobacteraceae and Pelagibacteraceae dominate.	Mainly Rhodobacteraceae (1621 ORFs), from various genera, most dominant being Planktomarina (895 ORFs).
	large	AGC_8808290	1230	Involved in urease metallocenter assembly. Binds nickel. Probably functions as a nickel donor during metallocenter assembly	UreE_C,UreE_N	Undefined	Among annotated sequences, Rhodobacterales, Rhodospirillales and Alteromonadales dominate.	Wide variety of bacteria, multiple Classes : gammaproteobacteria (526 ORFs), alphaproteobacteria (358 ORFs), Nitrospinia (277 ORFs),... Most dominant families are VA-1 (Nitrospinales order, 277 ORFs), Rhodobacteracea (150 ORFs) and Alteromonadaceae (113 ORFs).
	large	AGC_104708	22	urease accessory protein	cobW	Prasinodermaceae; Prasinoderma coloniale		Not applied
	large	AGC_17972926	11	cellular macromolecule catabolic process	PI3_PI4_kinase,Ribosomal_L41, Sod_Ni, ubiquitin	Bacillariaceae		Not applied
	large	AGC_15128084	251	urease activity	Amidohydro_1,DUF599,Fungal_trans,HMG_box,Urease_alpha,Urease_beta, Urease_gamma	Phaeocystaceae; Phaeocystis antarctica		Not applied
	large	AGC_26216502	11	superoxide dismutase copper chaperone activity	HMA,PB1,Sod_Cu,TPR_1,XPG_I_2	Prasinodermaceae; Prasinoderma singulare		Not applied
	large	AGC_27255931	831	Required for maturation of urease via the functional incorporation of the urease nickel metallocenter	UreF	Undefined	Blend of Proteobacteria, Cyanobacteria (Synechococcaceae) and Nitrospinae	Three main Classes, Gammaproteobacteria (486 ORFs, main genera Paraglaciecola and UBA9659)), Nitrospinia (264 ORFs, main genera Nitromaritima and LS-NOB)) and Cyanobacteriia (40 ORFs, main genera Parasynechococcus and Synechococcus_C).

<u>Table S2</u>: Functional and taxonomic annotations of all AGNOSTOS gene clusters (AGC) emerging from the redundancy analyses as particularly linked to δ^{60}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.