SUPPLEMENTAL MATERIAL

Data S1. Supplemental Methods

Bacterial DNA extraction and 16S rRNA sequencing

50 ng of DNA was used in polymerase chain reaction (PCR) amplification targeting the V3-V4 region of the 16S rRNA gene (using 341F/806R universal primers), and PCR products were purified using AMPure beads[22,23]. 100 ng of each library were pooled, gel-purified, and quantified using a bioanalyzer. 12 pM of the library mixture was spiked with 20% PhiX and run on a MiSeq (Illumina) platform. Raw reads were analyzed with QIIME2[24].

Data curation pipeline of the raw 16S rRNA sequences using QIIME2

Demultiplexed sequences were quality filtered with default parameters in qiime quality-filter q-score, namely, reads were trimmed after the first appearance of 3 basecalls with a PHRED score of 4 or less, and the entire read was removed if the read was truncated to less than 75% of the input sequence. Quality filtered forward-read sequences were denoised using Deblur with the default parameters[48]. Samples with less than 1,000 quality filtered reads were removed from downstream analysis. In order to remove reads aligned to chloroplast or mitochondrial genes, sequences were aligned using a classifier pretrained on the GreenGenes v13_8 database with 99% sequence homology using sklearn[49]. Sequences aligned to mitochondria or chloroplast were removed using filter-table --p-exclude (0.005% of the entire dataset). Overall, 44,776,283 sequences were generated for final analysis (median of 37,067 sequences per sample).

Data S2. Supplemental Results

Correlation of nitrate-reducing taxa summary score NO_3TSS and a priori individual taxa relative abundances with predicted gene abundance summary scores and ratios

A previous investigation using ORIGINS Wave 1 baseline data (n=281) found higher nitrate-reducing taxa summary score (NO_3TSS) associated with lower insulin resistance and plasma glucose in the full cohort, and lower systolic blood pressure in normotensive participants[13]. We replicated the operationalization of NO_3TSS and examined its correlation with the inferred gene abundance summary scores and ratios created in this study.

16S rRNA gene sequencing was performed per the HOMINGS (Human Oral Microbiome Identification using Next Generation Sequencing) methodology specifically designed for oral taxa to generate species-level information[22,23]. A customized BLAST program for taxonomic classification (ProbeSeq for HOMINGS), based on running the 16S rRNA sequence reads from Illumina sequencing against in-silico species-specific 16S rRNA-based oligonucleotide probes[23,50], was used to generate a final table of relative abundances of taxa species in that sample. The NO_3TSS was created by summing the standardized arcsine square root transformed relative abundance of taxa identified *a priori* to be associated with nitrate-reducing capacity[16,46].

NO_3TSS is optimized to include only taxa previously identified to be most associated with oral nitrate-reducing capacity whether directly or indirectly (as “helper” species) contributing to oral nitrate-reduction[16,46], and the small subset of a taxa is selected *a priori* and independently from the PICRUSt2 analyses. However, as gene abundances are inferred from the 16S rRNA gene sequences, some correlation with the taxa summary score NO_3TSS can be expected. The nitrate-reducing taxa summary score NO_3TSS was most strongly associated with the NO_2 product summary score (r=0.54, p<0.0001) and RD pathway summary score, and least associated with the DNRA pathway and NH_3 product summary score (Figure S3A). All nitrite generation-to-depletion ratios were moderately positively associated (r=0.41 to 0.44, p-value =0.0001) with the NO_3TSS (Figure S3B).

Among the individual *a priori* taxa that comprise the NO_3TSS, *Rothia denticariosa* and *Corynebacterium durum* showed the strongest associations with the NO_2 and NO gene abundance summary score (Figure S3A). *Actinomyces odontolyticus* did not have any associations with the individual gene abundance, summary scores or ratios. The nitrite generation-to depletion ratios were most strongly positively correlated to *A. naeslundii, C. durum, C. matruchotti*, and *R. dentocariosa (*Figure S3B). Only *Prevotella melaninogenica* was consistently inversely associated with the gene abundance ratios (Figure S3B). *Neisseria sicca* was not present in this dataset and not included in the taxa summary score.

In exploratory correlations of the taxa with individual gene abundances (Figure S3C), *Rothia dentocariosa* and *Corynebacterium durum* were the taxa most strongly associated with the *nar* and *nir* genes, and the *narK* nitrate/nitrite transporter gene. *Hemophilus parainfluenzae* had the strongest correlation with the *nap* nitrate reductase genes.

Table S1. Mean relative gene abundances (%) of the predicted 16 KEGG Orthologs (KO) corresponding to bacterial enzymes of interest in the NO_3-NO_2-NO pathway, in subgingival plaque samples among 764 participants in ORIGINS (Oral Infections, Glucose Intolerance, and Insulin Resistance Study).

Pathway	Product	Bacterial Gene	KO Identifier	Mean Relative Gene Abundance (%)*	Mean (SD) Absolute Gene Abundance*
Respiratory Denitrification (RD)	**NO_2**	*nar*		0.083970	
		narB	K00367[†]	0.000122	32 (185)
		narG, narZ, nxrA	K00370	0.027546	10457 (8389)
		narH, narY, nxrB	K00371	0.028143	10694 (8529)
		narI, narV	K00374	0.028159	10700 (8526)
	NO	*nir*		0.033071	
		nirK	K00368	0.033067	12569 (9821)
		nirS	K15864[†]	0.000004	1 (11)
Dissimilatory Nitrate Reduction to Ammonia (DNRA)	**NO_2**	*nap*		0.012145	
		napA	K02567	0.006082	2256 (2285)
		napB	K02568	0.006063	2250 (2286)
	NH_3	*nrf*		0.030303	
		nrfA	K03385	0.016998	6246 (4481)
		nrtH	K15876	0.013305	4864 (3892)
Assimilatory Nitrate Reduction (ANR)	**NO_2**	*nas*		0.001398	
		nasA	K00372[†]	0.000993	358 (652)
		nasB	K00360[†]	0.000405	150 (449)
	NH_3	*nir*		0.031157	
		nirA	K00366	0.011898	4243 (3286)
		nirB	K00362	0.009682	3905 (5514)
		nirD	K00363	0.009577	3872 (5502)
NO_3 and NO_2 Transport	-	*narK*	K02575	0.032667	12350 (9263)

[*] Relative gene abundance refers to the proportion of individual KEGG Orthologs (KO) counts over the total number of functional gene counts in the sample. Absolute count data should not be directly interpreted as we do not have information about total microbial load and biospecimen collection volume was not standardized. Counts are presented only to illustrate the distributions of the different KOs.

[†] KOs not detected in all participants; Prevalence of K15864 = 714 (93.5%), K00367 = 477 (62.4%), K00360 = 158 (20.7%), K00372 = 18 (2.4%).

K17877 and K10534 were not present in the dataset.

Abbreviations: **NO_3**, nitrate; **NO_2**, nitrite; **NO**, nitric oxide; **NH_3**, ammonia; **RD,** respiratory denitrification; **DNRA**, dissimilatory nitrate reduction to ammonia; **ANR**, assimilatory nitrate reduction; KEGG, Kyoto Encyclopedia of Genes and Genomes; KO: Kyoto Encyclopaedia of Genes and Genomes Orthologs

Table S2. Associations between potential confounders and quartiles of predicted gene abundances: A) NH_3 product summary score, B) NO vs. NH_3 nitrite generation-to-depletion ratio, and C) quartiles of composite cardiometabolic Z-score (CMZ)

Abbreviations: **NO**, nitric oxide; **NH_3**, ammonia; **BMI**, body mass index; **CMZ**: composite cardiometabolic score which averages the Z-scores for the individual cardiometabolic risk variables

A) NH_3 product summary score

Characteristic	**Q1 NH_3**	**Q2 NH_3**	**Q3 NH_3**	**Q4 NH_3**	**p-value**
Age (years)	33.13 (10.02)	31.58 (9.38)	31.02 (9.27)	30.93 (8.64)	0.0181
Sex					0.5948
Female	128 (67.02%)	136 (71.2%)	138 (72.25%)	139 (72.77%)	
Male	63 (32.98%)	55 (28.8%)	53 (27.75%)	52 (27.23%)	
Race/ethnicity					0.0042
Hispanic	57 (29.84%)	50 (26.32%)	52 (27.66%)	53 (28.04%)	
Non-Hispanic White	43 (22.51%)	58 (30.53%)	48 (25.53%)	68 (35.98%)	
Black	19 (9.95%)	28 (14.74%)	31 (16.49%)	33 (17.46%)	
Other	72 (37.7%)	54 (28.42%)	57 (30.32%)	35 (18.52%)	
Education					0.581
< Bachelor’s Degree	49 (26.63%)	43 (22.87%)	44 (23.66%)	35 (19.23%)	
Bachelor’s Degree	80 (43.48%)	94 (50%)	94 (50.54%)	97 (53.3%)	
> Bachelor’s Degree	55 (29.89%)	51 (27.13%)	48 (25.81%)	50 (27.47%)	
Smoking Status					0.7928
Never	151 (84.83%)	160 (87.43%)	164 (90.11%)	155 (86.59%)	
Former	12 (6.74%)	11 (6.01%)	10 (5.49%)	10 (5.59%)	
Current	15 (8.43%)	12 (6.56%)	8 (4.4%)	14 (7.82%)	
BMI ($kg/m^{2)}$	26.02 (5.73)	25.1 (5.45)	25.26 (5.92)	25.71 (6.1)	0.6844
Periodontal Disease					0.0902
None/Mild	123 (64.74%)	142 (75.13%)	142 (74.74%)	133 (70.74%)	
Moderate/Severe	67 (35.26%)	47 (24.87%)	48 (25.26%)	55 (29.26%)	

Values presented in mean (SD) or n (%). *P* values are for ANOVA F statistics or X^2 tests for differences in level of covariates between participants across increasing quartiles of predicted gene abundance NH_3 product summary score.

B) NO vs. NH_3 nitrite generation-to-depletion ratio

Characteristic	**Q1 NO vs. NH_3**	**Q2 NO vs. NH_3**	**Q3 NO vs. NH_3**	**Q4 NO vs. NH_3**	**p-value**
Age (years)	33.22 (9.96)	30.05 (8.47)	31.73 (9.58)	31.65 (9.2)	0.3163
Sex					0.6968
Female	541 (70.81%)	136 (71.2%)	141 (73.82%)	133 (69.63%)	
Male	223 (29.19%)	55 (28.8%)	50 (26.18%)	58 (30.37%)	
Race/ethnicity					0.0855
Hispanic	212 (27.97%)	64 (33.51%)	46 (24.47%)	48 (25.53%)	
Non-Hispanic White	217 (28.63%)	45 (23.56%)	59 (31.38%)	58 (30.85%)	
Black	111 (14.64%)	34 (17.8%)	24 (12.77%)	34 (18.09%)	
Other	218 (28.76%)	48 (25.13%)	59 (31.38%)	48 (25.53%)	
Education					0.0553
< Bachelor’s Degree	171 (23.11%)	53 (28.96%)	32 (17.58%)	48 (25.67%)	
Bachelor’s Degree	365 (49.32%)	84 (45.9%)	98 (53.85%)	96 (51.34%)	
> Bachelor’s Degree	204 (27.57%)	46 (25.14%)	52 (28.57%)	43 (22.99%)	
Smoking Status					0.801
Never	630 (87.26%)	153 (85.96%)	157 (87.71%)	161 (88.46%)	
Former	43 (5.96%)	10 (5.62%)	11 (6.15%)	8 (4.4%)	
Current	49 (6.79%)	15 (8.43%)	11 (6.15%)	13 (7.14%)	
BMI (kg/m$^{2)}$	25.52 (5.81)	26.4 (6.62)	25.22 (5.86)	25.63 (5.56)	0.0247
Periodontal Disease	55 (28.8%)	50 (26.18%)	58 (30.37%)	60 (31.41%)	0.1732
None/Mild	540 (71.33%)	123 (65.08%)	140 (74.07%)	141 (73.82%)	
Moderate/Severe	217 (28.67%)	66 (34.92%)	49 (25.93%)	50 (26.18%)	

Values presented in mean (SD) or n (%). *P* values are for ANOVA F statistics or X^2 tests for differences in level of covariates between participants across increasing quartiles of predicted gene abundance NO vs. NH_3 nitrite generation-to-depletion ratio.

C) Composite Cardiometabolic Z-score (CMZ)

Mean SD or %	Q1 CMZ	Q2 CMZ	Q3 CMZ	Q4 CMZ	p-value
Age (years)	29.27 (7.95)	29.34 (7.98)	31.31 (9.01)	36.74 (10.34)	<.0001
Sex					<.0001
Female	541 (70.81%)	158 (82.72%)	147 (76.96%)	126 (65.97%)	
Male	223 (29.19%)	33 (17.28%)	44 (23.04%)	65 (34.03%)	
Race/ethnicity					<.0001
Hispanic	212 (27.97%)	35 (18.42%)	54 (28.72%)	56 (29.63%)	
Non-Hispanic White	217 (28.63%)	81 (42.63%)	65 (34.57%)	46 (24.34%)	
Black	111 (14.64%)	15 (7.89%)	15 (7.98%)	29 (15.34%)	
Other	218 (28.76%)	59 (31.05%)	54 (28.72%)	58 (30.69%)	
Education					<.0001
< Bachelor's Degree	171 (23.11%)	22 (11.76%)	30 (16.3%)	45 (24.19%)	
Bachelor's Degree	365 (49.32%)	100 (53.48%)	104 (56.52%)	96 (51.61%)	
> Bachelor's Degree	204 (27.57%)	65 (34.76%)	50 (27.17%)	45 (24.19%)	
Smoking Status					0.3257
Never	630 (87.26%)	155 (85.64%)	160 (88.89%)	164 (90.61%)	
Former	43 (5.96%)	15 (8.29%)	8 (4.44%)	6 (3.31%)	
Current	49 (6.79%)	11 (6.08%)	12 (6.67%)	11 (6.08%)	
BMI (kg/$m^{2)}$	25.52 (5.81)	21.91 (3.16)	23.89 (4.06)	25.76 (4.98)	<.0001
Periodontal Disease					<.0001
None/Mild	540 (71.33%)	150 (78.95%)	144 (75.39%)	137 (73.26%)	
Moderate/Severe	217 (28.67%)	40 (21.05%)	47 (24.61%)	50 (26.74%)	

Values presented in mean (SD) or n (%). *P* values are for ANOVA F statistics or X^2 tests for differences in level of covariates between participants across increasing quartiles of composite cardiometabolic Z-score (CMZ).

Table S3. Results from Figure 2 – Association of predicted gene abundance pathway and product summary scores and ratios of nitrite/NO-generation-to-depletion with cardiometabolic risk Z-scores, in unadjusted (N=764), simple adjusted models (N=758) and fully adjusted models (N=705)

Metagenomic Score	Cardiometabolic risk variable (Z-score)	Unadjusted (N=764)			Adjusted (Simple) (N=758)			Adjusted (Full) (N=705)		
		Reg Coef	p-value	FDR q-value	Reg Coef	p-value	FDR q-value	Reg Coef	p-value	FDR q-value
Gene abundance pathway summary score										
RD	Composite	-0.07	0.0059	0.0221	-0.04	0.1000	0.1607	0.00	0.9943	0.9943
	Insulin	-0.10	0.0077	0.0252	-0.08	0.0326	0.0912	-0.03	0.4548	0.6964
	HOMA-iR	-0.09	0.0100	0.0307	-0.07	0.0534	0.1138	-0.02	0.6462	0.8051
	HbA1C	-0.07	0.0393	0.0767	-0.01	0.7648	0.8059	0.00	0.9205	0.9596
	FPG	-0.02	0.4999	0.5832	0.01	0.6698	0.7375	0.05	0.1239	0.3036
	DBP	-0.04	0.2880	0.3814	-0.01	0.8445	0.8682	0.02	0.5111	0.7366
	SBP	-0.08	0.0277	0.0591	-0.07	0.0396	0.0994	-0.03	0.3456	0.6048
ANR	Composite	0.01	0.8360	0.8716	0.02	0.3014	0.3938	0.03	0.1165	0.3036
	Insulin	-0.03	0.4188	0.5195	-0.02	0.6280	0.7156	-0.01	0.7516	0.8621
	HOMA-iR	-0.02	0.5215	0.5942	-0.01	0.8092	0.8437	0.00	0.9680	0.9882
	HbA1C	-0.02	0.6662	0.7174	0.04	0.2286	0.3155	0.05	0.1517	0.3458
	FPG	0.02	0.5239	0.5942	0.05	0.1688	0.2433	0.07	0.0313	0.2430
	DBP	0.05	0.2118	0.2965	0.06	0.0829	0.1424	0.05	0.1135	0.3036
	SBP	0.02	0.4917	0.5806	0.01	0.6681	0.7375	0.02	0.5339	0.7582
DNRA	Composite	0.02	0.3618	0.4604	0.04	0.0953	0.1557	0.04	0.0869	0.2838
	Insulin	0.03	0.4523	0.5472	0.02	0.4946	0.5771	0.01	0.6827	0.8061
	HOMA-iR	0.03	0.4679	0.5591	0.03	0.4227	0.5126	0.01	0.6768	0.8061
	HbA1C	0.07	0.0478	0.0885	0.06	0.0502	0.1093	0.07	0.0480	0.2447
	FPG	0.01	0.7922	0.8439	0.04	0.2472	0.3318	0.01	0.7909	0.8762
	DBP	0.00	0.8990	0.9124	0.03	0.4301	0.5140	0.04	0.2014	0.4111

	SBP	0.00	0.9016	0.9124	0.04	0.2452	0.3318	0.05	0.1155	0.3036
ANR + DNRA	Composite	0.03	0.2689	0.3661	0.05	0.0208	0.0669	0.06	0.0042	0.0817
	Insulin	0.00	0.9031	0.9124	0.00	0.9658	0.9757	0.00	0.9878	0.9943
	HOMA-iR	0.00	0.9896	0.9896	0.01	0.7255	0.7900	0.01	0.7566	0.8621
	HbA1C	0.06	0.1046	0.1681	0.09	0.0040	0.0303	0.10	0.0022	0.0531
	FPG	0.03	0.4142	0.5195	0.07	0.0365	0.0990	0.07	0.0353	0.2430
	DBP	0.05	0.1523	0.2180	0.08	0.0218	0.0669	0.09	0.0087	0.1048
	SBP	0.02	0.5275	0.5942	0.05	0.1625	0.2376	0.06	0.0599	0.2447
Gene abundance product summary score										
NO_2	Composite	-0.06	0.0142	0.0340	-0.03	0.2159	0.3023	0.01	0.6141	0.7993
	Insulin	-0.09	0.0118	0.0321	-0.07	0.0428	0.1023	-0.02	0.5586	0.7603
	HOMA-iR	-0.09	0.0162	0.0377	-0.06	0.0772	0.1408	-0.01	0.8047	0.8762
	HbA1C	-0.06	0.1127	0.1782	0.01	0.8505	0.8682	0.02	0.5475	0.7586
	FPG	-0.02	0.6548	0.7130	0.03	0.4053	0.5028	0.06	0.0554	0.2447
	DBP	-0.03	0.3454	0.4454	0.00	0.9996	0.9996	0.03	0.3582	0.6100
	SBP	-0.08	0.0334	0.0682	-0.06	0.0630	0.1234	-0.02	0.4990	0.7366
NO	Composite	-0.09	0.0004	0.0055	-0.06	0.0113	0.0481	-0.03	0.1943	0.4051
	Insulin	-0.12	0.0009	0.0068	-0.10	0.0057	0.0346	-0.06	0.0869	0.2838
	HOMA-iR	-0.12	0.0007	0.0067	-0.10	0.0066	0.0346	-0.05	0.1169	0.3036
	HbA1C	-0.10	0.0072	0.0243	-0.03	0.3140	0.3999	-0.02	0.5109	0.7366
	FPG	-0.07	0.0648	0.1114	-0.03	0.3142	0.3999	0.01	0.8235	0.8869
	DBP	-0.04	0.2381	0.3287	-0.01	0.7472	0.7960	0.01	0.8428	0.8978
	SBP	-0.07	0.0420	0.0791	-0.06	0.0708	0.1335	-0.03	0.3686	0.6123
NH_3	Composite	0.04	0.1470	0.2150	0.06	0.0089	0.0404	0.07	0.0010	0.0531
	Insulin	0.01	0.8067	0.8500	0.02	0.6663	0.7375	0.02	0.6490	0.8051
	HOMA-iR	0.02	0.6516	0.7130	0.03	0.4237	0.5126	0.03	0.3967	0.6479
	HbA1C	0.05	0.1323	0.1995	0.09	0.0038	0.0303	0.10	0.0021	0.0531
	FPG	0.05	0.1403	0.2083	0.09	0.0066	0.0346	0.09	0.0063	0.0885
	DBP	0.06	0.1238	0.1926	0.08	0.0214	0.0669	0.09	0.0055	0.0885

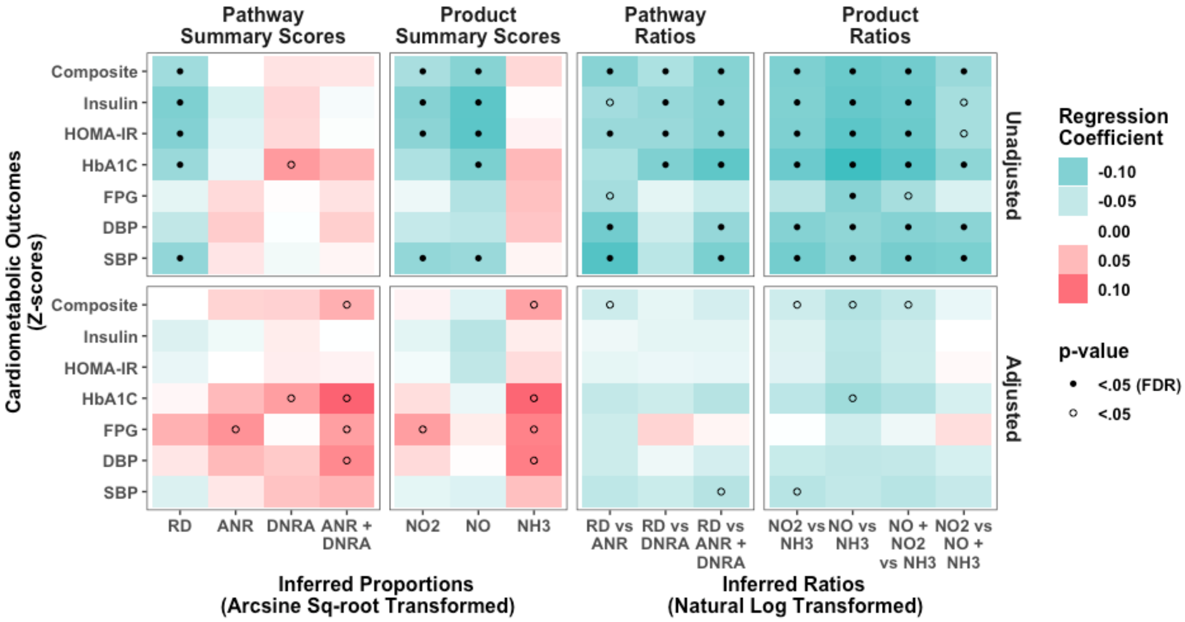
	SBP	0.02	0.5595	0.6230	0.04	0.2851	0.3776	0.05	0.1087	0.3036
Gene abundance pathway ratio										
RD vs ANR	Composite	-0.09	0.0005	0.0065	-0.07	0.0013	0.0303	-0.04	0.0434	0.2430
	Insulin	-0.07	0.0572	0.1000	-0.06	0.0860	0.1443	-0.01	0.7386	0.8617
	HOMA-iR	-0.07	0.0399	0.0767	-0.06	0.0675	0.1298	-0.02	0.6199	0.7993
	HbA1C	-0.07	0.0684	0.1136	-0.06	0.0610	0.1220	-0.05	0.1090	0.3036
	FPG	-0.06	0.0733	0.1197	-0.05	0.1463	0.2173	-0.04	0.2408	0.4720
	DBP	-0.10	0.0046	0.0189	-0.08	0.0152	0.0571	-0.04	0.1898	0.4043
	SBP	-0.12	0.0007	0.0067	-0.09	0.0043	0.0303	-0.06	0.0594	0.2447
RD vs DNRA	Composite	-0.06	0.0124	0.0328	-0.05	0.0297	0.0855	-0.02	0.2926	0.5233
	Insulin	-0.08	0.0317	0.0660	-0.06	0.0798	0.1408	-0.02	0.5496	0.7586
	HOMA-iR	-0.08	0.0353	0.0706	-0.06	0.0869	0.1443	-0.01	0.6624	0.8061
	HbA1C	-0.10	0.0071	0.0243	-0.05	0.1217	0.1893	-0.05	0.1781	0.3878
	FPG	-0.03	0.4461	0.5465	-0.02	0.5925	0.6832	0.03	0.4468	0.6964
	DBP	-0.04	0.3179	0.4154	-0.03	0.4356	0.5143	-0.01	0.6698	0.8061
	SBP	-0.05	0.1290	0.1976	-0.07	0.0384	0.0990	-0.05	0.1290	0.3083
RD vs ANR + DNRA	Composite	-0.09	0.0002	0.0047	-0.07	0.0019	0.0303	-0.04	0.0798	0.2838
	Insulin	-0.09	0.0133	0.0336	-0.07	0.0446	0.1023	-0.02	0.6039	0.7993
	HOMA-iR	-0.09	0.0117	0.0321	-0.07	0.0437	0.1023	-0.02	0.6366	0.8051
	HbA1C	-0.12	0.0010	0.0070	-0.07	0.0271	0.0804	-0.06	0.0578	0.2447
	FPG	-0.05	0.1535	0.2180	-0.03	0.3523	0.4426	0.00	0.9409	0.9706
	DBP	-0.08	0.0238	0.0531	-0.06	0.0805	0.1408	-0.04	0.2937	0.5233
	SBP	-0.10	0.0038	0.0163	-0.10	0.0032	0.0303	-0.06	0.0409	0.2430
Gene abundance product ratio										
NO_2 vs NH_3	Composite	-0.10	0.0001	0.0030	-0.08	0.0007	0.0236	-0.04	0.0392	0.2430
	Insulin	-0.10	0.0067	0.0243	-0.08	0.0215	0.0669	-0.03	0.4481	0.6964
	HOMA-iR	-0.10	0.0051	0.0202	-0.08	0.0194	0.0669	-0.03	0.4532	0.6964
	HbA1C	-0.11	0.0031	0.0146	-0.07	0.0377	0.0990	-0.06	0.0749	0.2838
	FPG	-0.07	0.0677	0.1136	-0.04	0.1912	0.2716	-0.01	0.7752	0.8732

	DBP	-0.09	0.0117	0.0321	-0.07	0.0454	0.1023	-0.05	0.1682	0.3747
	SBP	-0.11	0.0027	0.0145	-0.10	0.0036	0.0303	-0.06	0.0418	0.2430
NO vs NH_3	Composite	-0.12	<0.0001	0.0003	-0.10	<0.0001	0.0030	-0.07	0.0013	0.0531
	Insulin	-0.12	0.0007	0.0067	-0.10	0.0037	0.0303	-0.06	0.0772	0.2838
	HOMA-iR	-0.13	0.0003	0.0053	-0.11	0.0019	0.0303	-0.06	0.0586	0.2447
	HbA1C	-0.14	0.0001	0.0030	-0.09	0.0038	0.0303	-0.09	0.0096	0.1048
	FPG	-0.11	0.0028	0.0145	-0.09	0.0071	0.0346	-0.05	0.1233	0.3036
	DBP	-0.09	0.0142	0.0340	-0.07	0.0581	0.1185	-0.05	0.1226	0.3036
	SBP	-0.10	0.0083	0.0261	-0.08	0.0133	0.0523	-0.05	0.0832	0.2838
NO + NO_2 vs NH_3	Composite	-0.11	<0.0001	0.0010	-0.09	0.0002	0.0088	-0.05	0.0121	0.1182
	Insulin	-0.11	0.0022	0.0128	-0.09	0.0091	0.0404	-0.04	0.2506	0.4816
	HOMA-iR	-0.11	0.0015	0.0097	-0.10	0.0071	0.0346	-0.04	0.2387	0.4720
	HbA1C	-0.12	0.0009	0.0068	-0.08	0.0162	0.0590	-0.07	0.0360	0.2430
	FPG	-0.08	0.0275	0.0591	-0.06	0.0779	0.1408	-0.02	0.4930	0.7366
	DBP	-0.09	0.0114	0.0321	-0.07	0.0459	0.1023	-0.05	0.1493	0.3458
	SBP	-0.11	0.0031	0.0146	-0.10	0.0042	0.0303	-0.06	0.0446	0.2430
NO_2 vs NO + NH_3	Composite	-0.08	0.0016	0.0100	-0.06	0.0129	0.0523	-0.02	0.3610	0.6100
	Insulin	-0.07	0.0542	0.0965	-0.06	0.1199	0.1893	0.01	0.8611	0.9074
	HOMA-iR	-0.07	0.0498	0.0905	-0.05	0.1319	0.2020	0.01	0.8004	0.8762
	HbA1C	-0.08	0.0211	0.0480	-0.05	0.1463	0.2173	-0.04	0.2797	0.5172
	FPG	-0.04	0.2751	0.3693	-0.01	0.7380	0.7948	0.02	0.5891	0.7909
	DBP	-0.09	0.0134	0.0336	-0.07	0.0577	0.1185	-0.04	0.2772	0.5172
	SBP	-0.11	0.0033	0.0148	-0.09	0.0066	0.0346	-0.05	0.0912	0.2883

Abbreviations: **NO_3**, nitrate; **NO_2**, nitrite; **NO**, nitric oxide; **NH_3**, ammonia; **RD,** respiratory denitrification; **DNRA**, dissimilatory nitrate reduction to ammonia; **ANR**, assimilatory nitrate reduction; **CMZ**: composite cardiometabolic score which averages the Z-scores for the individual cardiometabolic risk variables; **HOMA-IR**: homeostatic model assessment of insulin resistance, **HbA1c**: haemoglobin A1c; **FPG**: fasting plasma glucose; **DBP**: diastolic blood pressure; **SBP**: systolic blood pressure; **FDR**: false discovery rate.

Simple adjusted multivariable models controlling for sex, age, race/ethinicity.
Fully adjusted multivariable models controlling for sex, age, race/ethnicity, education, smoking status, body mass index and periodontal disease
Summary scores were arcsine square root transformed and ratios of nitrite generation-to-depletion underwent natural log-transformation before use in linear regression analyses. Results are presented as the change in the dependent variable for a 1 standard deviation change in the independent variable.

Figure S1.



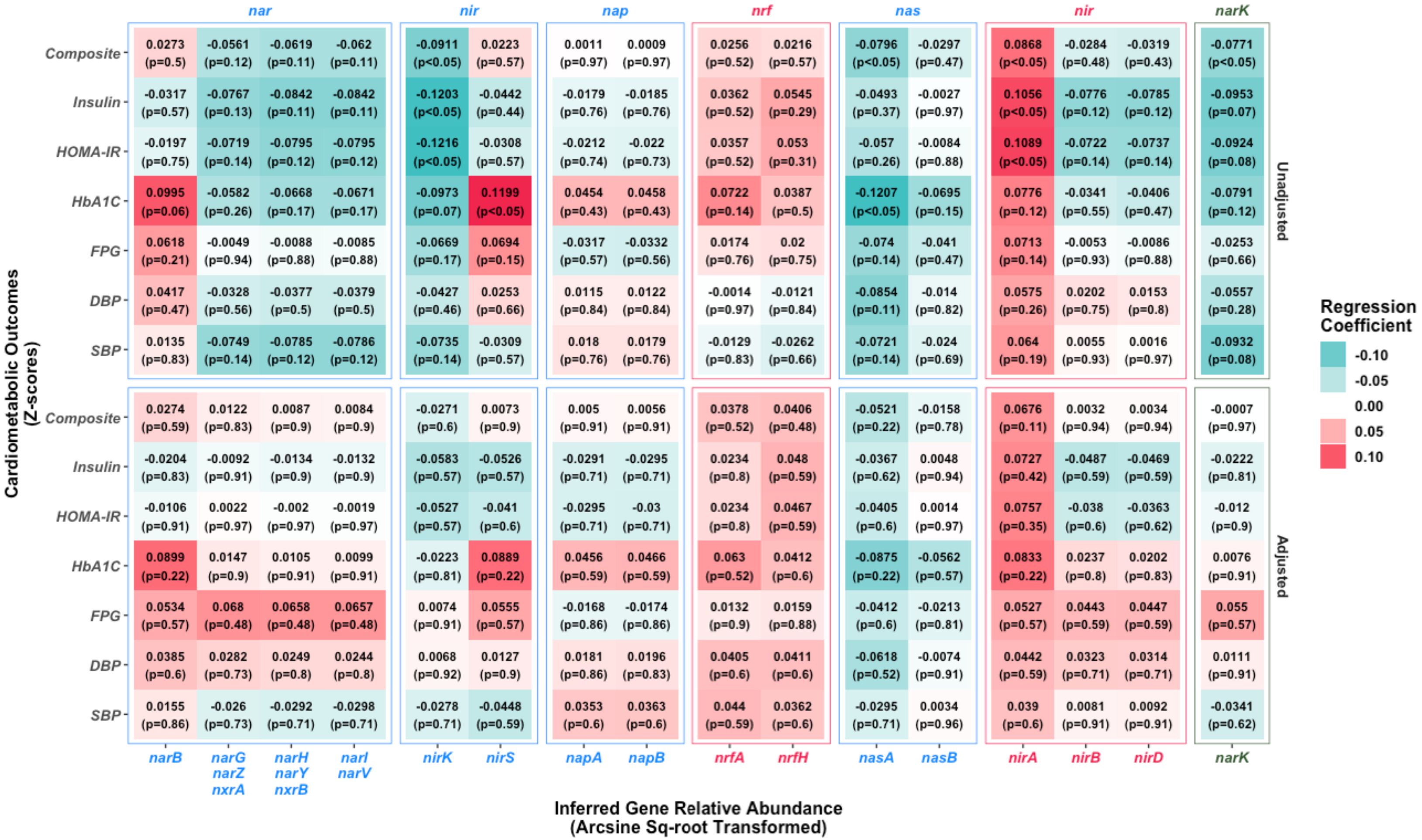
Association between select measures of nitrogen metabolism and cardiometabolic risk Z-scores, unadjusted and fully adjusted for sex, age, race/ethnicity, education, smoking status, body mass index, and periodontal disease. Results from the within-mouth average of predicted gene abundances across samples from shallow and deep sites in n=772 participants in the Oral Infections, Glucose Intolerance and Insulin Resistance Study (ORIGINS).

This heatmap represents the beta coefficients (change in cardiometabolic risk Z-score per 1 SD increase in metagenomic variable) from i) unadjusted (upper panels) and ii) fully adjusted multivariable linear regression models (lower panels) regressing cardiometabolic Z-scores on the following measures of nitrogen metabolism. 2A) Abundance of microbial genes in the following 3 pathways were considered: respiratory denitrification (RD); dissimilatory nitrate reduction to ammonia (DNRA); and assimilatory nitrate reduction (ANR); 2B) Abundance of microbial genes for reductase enzymes that produce the following biochemical products: (NO_2, NO, and NH_3); 2C) Nitrite generation-vs-depletion ratios of microbial gene abundances for pathways (RD vs. ANR, RD vs. DNRA, RD vs. ANR+DNRA) or products (NO_2 vs. NH_3, NO vs. NH_3, NO+NO_2 vs. NH_3, NO_2 vs. NO + NH_3). Green represents inverse associations, and red represents positive associations. Darker colors represent a larger effect estimate.

Within-mouth average gene abundances were obtained from both shallow and deep sites by weighting samples by the percentage of shallow (< 4mm) vs. deep (≥ 4mm) probing depths in the mouth, respectively. Participants with only shallow or disease sample were assigned a weight of 100% for that sample. Three participants were missing percentage of probing depths measurements and excluded from the analyses.

Abbreviations: **NO_3**, nitrate; **NO_2**, nitrite; **NO**, nitric oxide; **NH_3**, ammonia; **RD,** respiratory denitrification; **DNRA**, dissimilatory nitrate reduction to ammonia; **ANR**, assimilatory nitrate reduction; **CMZ**: composite cardiometabolic score which averages the Z-scores for the individual cardiometabolic risk variables; **HOMA-IR**: homeostatic model assessment of insulin resistance, **HbA1c**: haemoglobin A1c; **FPG**: fasting plasma glucose; **DBP**: diastolic blood pressure; **SBP**: systolic blood pressure; **FDR**: false discovery rate.

Figure S2.



	nar				nir		nap		nrf		nas		nir			narK
Unadjusted	narB	narG narZ nxrA	narH narY nxrB	narI narV	nirK	nirS	napA	napB	nrfA	nrfH	nasA	nasB	nirA	nirB	nirD	narK
Composite	0.0273 (p=0.5)	-0.0561 (p=0.12)	-0.0619 (p=0.11)	-0.062 (p=0.11)	-0.0911 (p<0.05)	0.0223 (p=0.57)	0.0011 (p=0.97)	0.0009 (p=0.97)	0.0256 (p=0.52)	0.0216 (p=0.57)	-0.0796 (p<0.05)	-0.0297 (p=0.47)	0.0868 (p<0.05)	-0.0284 (p=0.48)	-0.0319 (p=0.43)	-0.0771 (p<0.05)
Insulin	-0.0317 (p=0.57)	-0.0767 (p=0.13)	-0.0842 (p=0.11)	-0.0842 (p=0.11)	-0.1203 (p<0.05)	-0.0442 (p=0.44)	-0.0179 (p=0.76)	-0.0185 (p=0.76)	0.0362 (p=0.52)	0.0545 (p=0.29)	-0.0493 (p=0.37)	-0.0027 (p=0.97)	0.1056 (p<0.05)	-0.0776 (p=0.12)	-0.0785 (p=0.12)	-0.0953 (p=0.07)
HOMA-IR	-0.0197 (p=0.75)	-0.0719 (p=0.14)	-0.0795 (p=0.12)	-0.0795 (p=0.12)	-0.1216 (p<0.05)	-0.0308 (p=0.57)	-0.0212 (p=0.74)	-0.022 (p=0.73)	0.0357 (p=0.52)	0.053 (p=0.31)	-0.057 (p=0.26)	-0.0084 (p=0.88)	0.1089 (p<0.05)	-0.0722 (p=0.14)	-0.0737 (p=0.14)	-0.0924 (p=0.08)
HbA1C	0.0995 (p=0.06)	-0.0582 (p=0.26)	-0.0668 (p=0.17)	-0.0671 (p=0.17)	-0.0973 (p=0.07)	0.1199 (p<0.05)	0.0454 (p=0.43)	0.0458 (p=0.43)	0.0722 (p=0.14)	0.0387 (p=0.5)	-0.1207 (p<0.05)	-0.0695 (p=0.15)	0.0776 (p=0.12)	-0.0341 (p=0.55)	-0.0406 (p=0.47)	-0.0791 (p=0.12)
FPG	0.0618 (p=0.21)	-0.0049 (p=0.94)	-0.0088 (p=0.88)	-0.0085 (p=0.88)	-0.0669 (p=0.17)	0.0694 (p=0.15)	-0.0317 (p=0.57)	-0.0332 (p=0.56)	0.0174 (p=0.76)	0.02 (p=0.75)	-0.074 (p=0.14)	-0.041 (p=0.47)	0.0713 (p=0.14)	-0.0053 (p=0.93)	-0.0086 (p=0.88)	-0.0253 (p=0.66)
DBP	0.0417 (p=0.47)	-0.0328 (p=0.56)	-0.0377 (p=0.5)	-0.0379 (p=0.5)	-0.0427 (p=0.46)	0.0253 (p=0.66)	0.0115 (p=0.84)	0.0122 (p=0.84)	-0.0014 (p=0.97)	-0.0121 (p=0.84)	-0.0854 (p=0.11)	-0.014 (p=0.82)	0.0575 (p=0.26)	0.0202 (p=0.75)	0.0153 (p=0.8)	-0.0557 (p=0.28)
SBP	0.0135 (p=0.83)	-0.0749 (p=0.14)	-0.0785 (p=0.12)	-0.0786 (p=0.12)	-0.0735 (p=0.14)	-0.0309 (p=0.57)	0.018 (p=0.76)	0.0179 (p=0.76)	-0.0129 (p=0.83)	-0.0262 (p=0.66)	-0.0721 (p=0.14)	-0.024 (p=0.69)	0.064 (p=0.19)	0.0055 (p=0.93)	0.0016 (p=0.97)	-0.0932 (p=0.08)

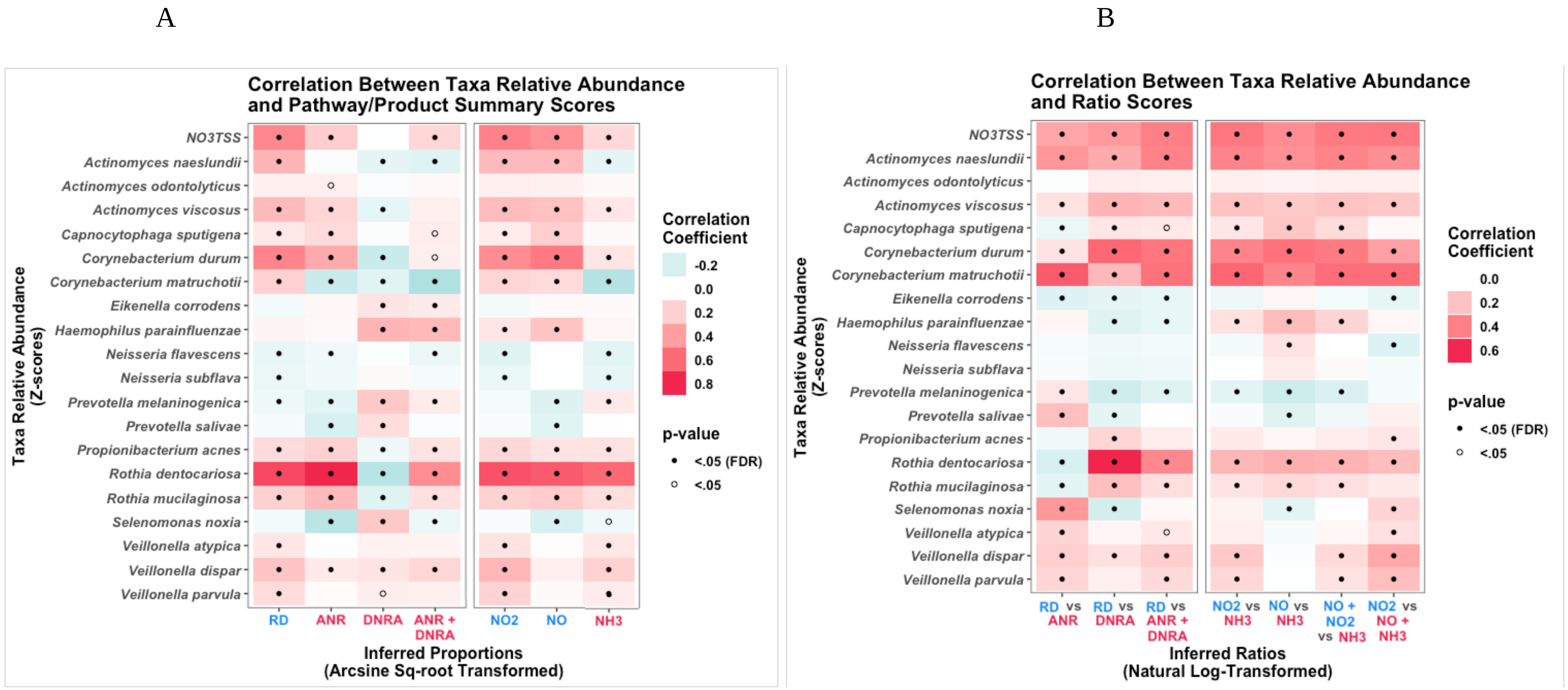
	nar				nir		nap		nrf		nas		nir			narK
Adjusted	narB	narG narZ nxrA	narH narY nxrB	narI narV	nirK	nirS	napA	napB	nrfA	nrfH	nasA	nasB	nirA	nirB	nirD	narK
Composite	0.0274 (p=0.59)	0.0122 (p=0.83)	0.0087 (p=0.9)	0.0084 (p=0.9)	-0.0271 (p=0.6)	0.0073 (p=0.9)	0.005 (p=0.91)	0.0056 (p=0.91)	0.0378 (p=0.52)	0.0406 (p=0.48)	-0.0521 (p=0.22)	-0.0158 (p=0.78)	0.0676 (p=0.11)	0.0032 (p=0.94)	0.0034 (p=0.94)	-0.0007 (p=0.97)
Insulin	-0.0204 (p=0.83)	-0.0092 (p=0.91)	-0.0134 (p=0.9)	-0.0132 (p=0.9)	-0.0583 (p=0.57)	-0.0526 (p=0.57)	-0.0291 (p=0.71)	-0.0295 (p=0.71)	0.0234 (p=0.8)	0.048 (p=0.59)	-0.0367 (p=0.62)	0.0048 (p=0.94)	0.0727 (p=0.42)	-0.0487 (p=0.59)	-0.0469 (p=0.59)	-0.0222 (p=0.81)
HOMA-IR	-0.0106 (p=0.91)	0.0022 (p=0.97)	-0.002 (p=0.97)	-0.0019 (p=0.97)	-0.0527 (p=0.57)	-0.041 (p=0.6)	-0.0295 (p=0.71)	-0.03 (p=0.71)	0.0234 (p=0.8)	0.0467 (p=0.59)	-0.0405 (p=0.6)	0.0014 (p=0.97)	0.0757 (p=0.35)	-0.038 (p=0.6)	-0.0363 (p=0.62)	-0.012 (p=0.9)
HbA1C	0.0899 (p=0.22)	0.0147 (p=0.9)	0.0105 (p=0.91)	0.0099 (p=0.91)	-0.0223 (p=0.81)	0.0889 (p=0.22)	0.0456 (p=0.59)	0.0466 (p=0.59)	0.063 (p=0.52)	0.0412 (p=0.6)	-0.0875 (p=0.22)	-0.0562 (p=0.57)	0.0833 (p=0.22)	0.0237 (p=0.8)	0.0202 (p=0.83)	0.0076 (p=0.91)
FPG	0.0534 (p=0.57)	0.068 (p=0.48)	0.0658 (p=0.48)	0.0657 (p=0.48)	0.0074 (p=0.91)	0.0555 (p=0.57)	-0.0168 (p=0.86)	-0.0174 (p=0.86)	0.0132 (p=0.9)	0.0159 (p=0.88)	-0.0412 (p=0.6)	-0.0213 (p=0.81)	0.0527 (p=0.57)	0.0443 (p=0.59)	0.0447 (p=0.59)	0.055 (p=0.57)
DBP	0.0385 (p=0.6)	0.0282 (p=0.73)	0.0249 (p=0.8)	0.0244 (p=0.8)	0.0068 (p=0.92)	0.0127 (p=0.9)	0.0181 (p=0.86)	0.0196 (p=0.83)	0.0405 (p=0.6)	0.0411 (p=0.6)	-0.0618 (p=0.52)	-0.0074 (p=0.91)	0.0442 (p=0.59)	0.0323 (p=0.71)	0.0314 (p=0.71)	0.0111 (p=0.91)
SBP	0.0155 (p=0.86)	-0.026 (p=0.73)	-0.0292 (p=0.71)	-0.0298 (p=0.71)	-0.0278 (p=0.71)	-0.0448 (p=0.59)	0.0353 (p=0.6)	0.0363 (p=0.6)	0.044 (p=0.59)	0.0362 (p=0.6)	-0.0295 (p=0.71)	0.0034 (p=0.96)	0.039 (p=0.6)	0.0081 (p=0.91)	0.0092 (p=0.91)	-0.0341 (p=0.62)

Exploratory analyses of the 16 individual natural-log transformed predicted relative gene abundances and cardiometabolic risk Z-scores, unadjusted (N=764) and fully adjusted for sex, age, race/ethnicity, education smoking status, body mass index, and periodontal disease (N= 705)

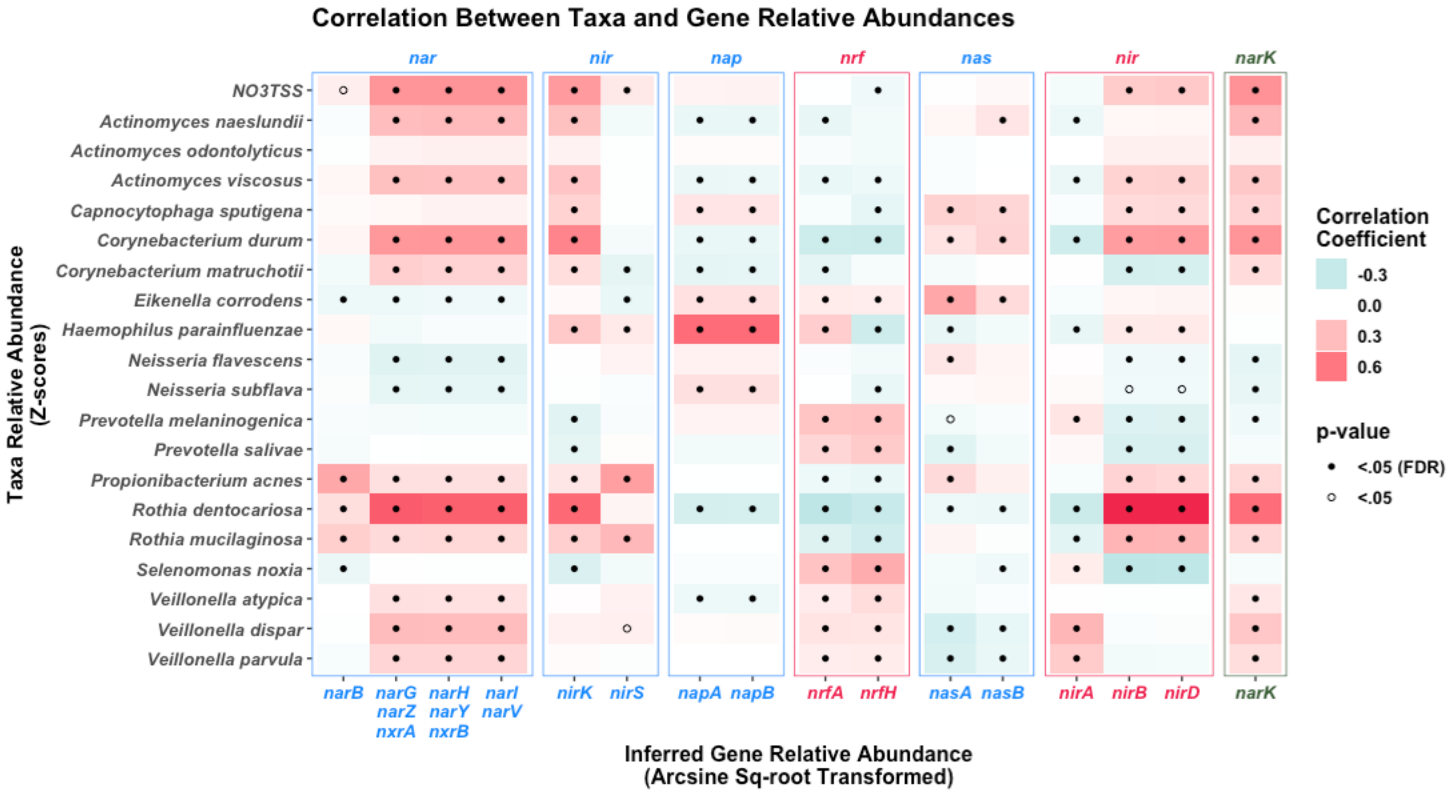
Note: Regression coefficients are scaled to present the change in the dependent variable for a 1 standard deviation change in the independent variable.

Abbreviations: CMZ: composite cardiometabolic score which averages the Z-scores for the individual cardiometabolic risk variables; HOMA-IR: homeostatic model assessment of insulin resistance, HbA1c: haemoglobin A1c; FPG: fasting plasma glucose; DBP: diastolic blood pressure; SBP: systolic blood pressure

Figure S3.


A
B
Correlation Between Taxa Relative Abundance and Pathway/Product Summary Scores
Taxa Relative Abundance (Z-scores)
NO3TSS
Actinomyces naeslundii
Actinomyces odontolyticus
Actinomyces viscosus
Capnocytophaga sputigena
Corynebacterium durum
Corynebacterium matruchotii
Eikenella corrodens
Haemophilus parainfluenzae
Neisseria flavescens
Neisseria subflava
Prevotella melaninogenica
Prevotella salivae
Propionibacterium acnes
Rothia dentocariosa
Rothia mucilaginosa
Selenomonas noxia
Veillonella atypica
Veillonella dispar
Veillonella parvula
RD
ANR
DNRA
ANR + DNRA
NO2
NO
NH3
Inferred Proportions (Arcsine Sq-root Transformed)
Correlation Coefficient
-0.2
0.0
0.2
0.4
0.6
0.8
p-value
<.05 (FDR)
<.05
Correlation Between Taxa Relative Abundance and Ratio Scores
Taxa Relative Abundance (Z-scores)
RD vs ANR
RD vs DNRA
RD vs ANR + DNRA
NO2 vs NH3
NO vs NH3
NO + NO2 vs NH3
NO2 vs NO + NH3
Inferred Ratios (Natural Log-Transformed)
Correlation Coefficient
0.0
0.2
0.4
0.6
p-value
<.05 (FDR)
<.05

C



Correlation matrices of the nitrate-reducing taxa summary score (NO$_3$TSS) and individual *a priori* taxa, with the A) gene abundance pathway and biochemical product summary scores, B) nitrite generation-to-depletion gene abundance ratios, and C) individual relative gene abundances

Note: Neisseria sicca was not present in this dataset.

Abbreviations: **NO_3TSS**, nitrate-reducing taxa summary score; **NO_3**, nitrate; **NO_2**, nitrite; **NO**, nitric oxide; **NH_3**, ammonia; **RD,** respiratory denitrification; **DNRA**, dissimilatory nitrate reduction to ammonia; **ANR**, assimilatory nitrate reduction; **FDR**: false discovery rate.