Online Data Supplement

Upper Respiratory Dysbiosis with a Facultative-Dominated Ecotype in Advanced Lung Disease and Dynamic Change after Lung Transplant

Aurea Simon-Soro, Michael B. Sohn, John E. McGinniss, Ize Imai, Melanie C. Brown, Vincent R. Knecht, Aubrey Bailey, Erik L. Clarke, Edward Cantu, Hongzhe Li, Kyle Bittinger, Joshua M. Diamond, Jason D. Christie, Frederic D. Bushman, Ronald G. Collman

Table E1. Clinical and demographical data of subjects.

SUBJECT.ID	AGE	GENDER	DISEASE	NUMBER SAMPLES	TRANSPLANT TYPE	OUTCOME	LAS
HUP3B10	65	M	Healthy	1	None		
HUP3C04	21	M	Healthy	1	None		
HUP3C05	28	M	Healthy	1	None		
HUP3C06	23	M	Healthy	1	None		
HUP3C07	40	M	Healthy	1	None		
HUP3D02	37	F	Healthy	1	None		
HUP3D03	51	F	Healthy	1	None		
HUP3D04	32	M	Healthy	1	None		
HUP3D06	41	M	Healthy	1	None		
107OW	34	M	Healthy	1	None		
108OW	49	F	Healthy	1	None		
109OW	38	F	Healthy	1	None		
113OW	33	F	Healthy	1	None		
114OW	37	F	Healthy	1	None		
1000798	64	M	IPF	1	PreTx		36.7719
1000932	60	F	COPD	1	PreTx		39.017
1000936	39	F	LAM	1	PreTx		34.091
1000946	61	F	COPD	1	PreTx		32.5753
1000990	70	F	COPD	1	PreTx		32.1604
1001027	66	F	IPF	1	PreTx		51.7943
1001035	46	F	Sarcoidosis	1	PreTx		35.0347
1001045	65	M	COPD	1	PreTx		33
1001049	52	M	IPF	1	PreTx		37.2947
1001061	57	F	COPD	1	PreTx		34.48
1001062	59	F	IPF	1	PreTx		41.6194
1001064	69	F	PH/PAH	1	PreTx		38.1428
1001066	59	F	OB	1	PreTx		45.429
1001067	64	M	IPF	1	PreTx		42.42
1001068	65	M	COPD	1	PreTx		33.346
1001087	44	F	LAM	1	PreTx		35.1469
1001107	72	M	IPF	1	PreTx		47.0169
1001109	59	M	IPF	1	PreTx		59.7239
1001110	60	F	Sarcoidosis	1	PreTx		NA
1001117	54	M	Sarcoidosis	1	PreTx		37.2161
1001122	59	M	IPF	1	PreTx		86.9722
1001127	67	F	Sarcoidosis	1	PreTx		NA

641000068	64	M	Sarcoidosis	3	Bilateral	PGD	39.8396
641000074	70	M	COPD	3	Single	No	33.4759
641000079	66	F	Non-IPF	3	Single	ACR A2 at 6w, BOS	42.0325
641000084	50	M	IPF	3	Bilateral	PGD	42.8918
641000085	60	F	Non-IPF	3	Bilateral	No	35.8376
641000771	75	M	COPD	3	Single	ACR A2 at 6w, PGD	31.8742
641000777	58	F	COPD	3	Bilateral	BOS, PGD	34.9693
641000785	55	F	Re-Transplanted	3	Single	ACR A2 at 3m	42.5993
641000802	52	F	IPF	3	Single	No	37.9103
641000817	58	F	COPD	3	Single	No	31.5751
641000827	53	F	COPD	3	Bilateral	PGD	41.8639
641000840	54	M	Pneumonitis	3	Bilateral	No	36.3642
641000845	56	F	IPF	3	Bilateral	ACR A2 at 6w and 3m	40.4968
641000847	65	M	IPF	3	Single	ACR A2 at 6w and 3m	42.1095
641000851	66	F	COPD	3	Bilateral	PGD	39.8834
641000854	66	F	COPD	2	Single	PGD	33.0739
641000857	61	M	Non-IPF	3	Bilateral	PGD	76.684
641000879	51	M	Non-IPF	3	Single	No	37.2935
641000889	58	M	IPF	2	Bilateral	PGD	89.5462
641000890	60	M	Non-IPF	3	Single	ACR A2 at 6w	40.1042
641000891	35	F	Non-IPF	3	Bilateral	No	40.1231
641000904	53	M	Non-IPF	3	Bilateral	PGD	91.1559
641000912	70	M	COPD	3	Single	Death	32.3975
641000914	64	M	COPD	3	Bilateral	No	46.4286
641000921	47	M	COPD	3	Bilateral	No	33.4402
641000929	21	M	Non-IPF	3	Bilateral	No	90.5294
64710000057	61	M	IPF	3	Single	BOS	47.3997
64710000088	57	F	Non-IPF	3	Single	ACR A2 at 3m	34.7478
64710000095	66	M	IPF	3	Single	No	89.6879
64710000098	65	M	IPF	3	Single	ACR A2 at 6w, PGD	35.4774
64710000099	57	F	Non-IPF	3	Bilateral	PGD, Death	65.1491
64710000101	65	F	COPD	3	Single	No	35.8442
64710000105	60	F	Non-IPF	3	Single	PGD, Death	55.3481

Abbreviations:
Gender: M= Male, F= Female
Disease: IPF= Idiopathic pulmonary fibrosis, COPD= Chronic obstructive pulmonary disease, LAM= lymphangioleiomyomatosis, PH/PAH= Pulmonary hypertension/Pulmonary arterial hypertension, OB= Obliterative bronchiolitis
Outcomes: PGD= Primary graft dysfunction, ACR= Acute cellular rejection, BOS=
LAS: Lung allocation score; NA= not available

Table E2. Bacterial respiration phenotypes of taxa above 1% abundances.

GENUS	CELL RESPIRATION	**REFERENCE**
Abiotrophia	Facultative	(Kawamura et al., 1995)
Acinetobacter	Aerobe	(Bouvet and Grimont, 1986)
Actinobacillus	Facultative	(Olsen and Møller, 2015)
Actinomyces	Facultative	(Hall, 2005)
Aggregatibacter	Facultative	(Norskov-Lauritsen, 2006)
Agrobacterium	Aerobe	(Sawada et al., 1993)
Alicyclobacillus	Aerobe	(Wisotzkey et al., 1992)
Anaerococcus	Anaerobe	(Ezaki and Ohkusu, 2015)
Anaerovorax	Anaerobe	(Matthies et al., 2000)
Anoxybacillus	Facultative	(Pikuta, 2003)
Arthrobacter	Aerobe	(Koch et al., 1995)
Atopobium	Anaerobe	(Cools et al., 2014)
Bacillus	Facultative	(Logan and Vos, 2015)
Bifidobacterium	Anaerobe	(Crociani et al., 1996)
Bilophila	Anaerobe	(Baron, 2015)
Bradyrhizobium	Aerobe	(Jordan, 1982)
Brevibacterium	Aerobe	(Trujillo and Goodfellow, 2015)
Bulleidia	Anaerobe	(Downes et al., 2000)
Butyrivibrio	Anaerobe	(Willems and Collins, 2015a)
Caldicellulosiruptor	Anaerobe	(Rainey et al., 1994)
Campylobacter	Anaerobic	(Etoh et al., 1993)
Capnocytophaga	Facultative	(London et al., 1985)
Cardiobacterium	Facultative	(Paster and Dewhirst, 2015)
Catonella	Anaerobe	(Moore and Moore, 1994)
Chryseobacterium	Aerobe	(Bernardet et al., 2015)
Cloacibacterium	Facultative	(Allen, 2006)
Clostridium	Anaerobe	(Rainey et al., 2015)
Conchiformibius	Aerobe	(Xie and Yokota, 2005)
Coprococcus	Anaerobe	(Holdeman and Moore, 1974)
Corynebacterium	Facultative	(Bernard and Funke, 2015)
Curtobacterium	Aerobe	(Yamada and Komagata, 1972)
Delftia	Aerobe	(Wen et al., 1999)
Dermacoccus	Aerobe	(Stackebrandt et al., 1995)
Desulfobulbus	Anaerobe	(Kuever et al., 2015)
Desulfomicrobium	Anaerobe	(Langendijk et al., 2001)
Dialister	Anaerobe	(Downes, 2003)

Eikenella	Facultative	(Jackson and Goodman, 1972)
Enhydrobacter	Facultative	(Staley et al., 1987)
Enterococcus	Facultative	(Schleifer and Kilpper-Balz, 1984)
Erwinia	Facultative	(Hauben and Swings, 2015)
Exiguobacterium	Facultative	(Collins, 2015)
Filifactor	Anaerobe	(Jalava and Eerola, 2015)
Finegoldia	Anaerobe	(Ezaki, 2015a)
Flavobacterium	Aerobe	(Bernardet and Bowman, 2015)
Fusobacterium	Anaerobe	(Gharbia et al., 2015)
Gemella	Facultative	(Collins and Falsen, 2015)
Geobacillus	Aerobe	(Ivanov et al., 2001)
Gordonia	Aerobe	(Goodfellow et al., 2015)
Granulicatella	Facultative	(Lawson, 2015)
Haemophilus	Facultative	(Kilian, 2015)
Herbaspirillum	Anaerobe	(Baldani et al., 2015)
Janthinobacterium	Aerobe	(Gillis and Logan, 2015)
Jonquetella	Anaerobe	(Jumas-Bilak et al., 2007)
Kingella	Aerobe	(Weyant, 2015)
Lactobacillus	Facultative	(Hammes and Hertel, 2015)
Lactococcus	Facultative	(Teuber, 2015)
Lautropia	Facultative	(Gerner-Smidt, 2015)
Leptotrichia	Anaerobe	(Eisenberg et al., 2018a)
Leuconostoc	Facultative	(Holzapfel et al., 2015a)
Luteibacter	Aerobe	(Johansen, 2005)
Macrococcus	Facultative	(Schleifer, 2015)
Megasphaera	Anaerobe	(Marchandin et al., 2015)
Meiothermus	Aerobe	(Nobre et al., 1996)
Methylobacterium	Aerobe	(Green, 2015)
Microbacterium	Aerobe	(Suzuki and Hamada, 2015)
Micrococcus	Aerobe	(Busse, 2015)
Mobiluncus	Anaerobe	(Hoyles and McCartney, 2015)
Modestobacter	Aerobe	(Normand and Benson, 2015)
Mogibacterium	Anaerobe	(The Editorial Board, 2015a)
Moraxella	Aerobe	(Juni and Bøvre, 2015)
Moryella	Anaerobe	(Carlier, 2015a)
Mycobacterium	Aerobe	(Magee and Ward, 2015)
Mycoplasma	Facultative	(Brown et al., 2018)
Neisseria	Aerobe	(Tønjum, 2015)
Nesterenkonia	Aerobe	(Stackebrandt, 2015)

Ochrobactrum	Aerobic	(Kämpfer et al., 2018)
Oribacterium	Anaerobe	(Carlier, 2015b)
Paenibacillus	Facultative	(Priest, 2015)
Paludibacter	Anaerobe	(Ueki, 2006)
Paracoccus	Aerobe	(van Spanning et al., 2015)
Parvimonas	Anaerobe	(The Editorial Board, 2015b)
Pediococcus	Facultative	(Holzapfel et al., 2015b)
Peptococcus	Anaerobe	(Ezaki, 2015b)
Peptoniphilus	Anaerobe	(Ezaki and Kawamura, 2015)
Peptostreptococcus	Anaerobe	(Ezaki, 2015c)
Phenylobacterium	Aerobe	(Eberspächer, 2015)
Porphyromonas	Anaerobe	(Summanen and Finegold, 2015)
Prevotella	Anaerobe	(Shah et al., 2015)
Propionibacterium	Anaerobe	(Patrick and McDowell, 2015)
Propionivibrio	Anaerobe	(The Editorial Board, 2015c)
Pseudomonas	Aerobe	(Palleroni, 2015a)
Pseudoramibacter	Anaerobe	(Willems and Collins, 2015b)
Psychrobacter	Aerobe	(Juni, 2015)
Pyramidobacter	Anaerobe	(Downes et al., 2009)
Ralstonia	Aerobe	(Yabuuchi et al., 2015)
Rheinheimera	Aerobe	(Brettar et al., 2002)
Rhizobium	Aerobe	(Kuykendall et al., 2015)
Rhodobacter	Anaerobe	(Imhoff, 2015)
Rhodococcus	Aerobe	(Jones and Goodfellow, 2015)
Roseomonas	Aerobe	(Weyant and Whitney, 2015)
Rothia	Facultative	(Austin, 2015)
Rubellimicrobium	Aerobe	(Denner, 2006)
Ruminococcus	Anaerobe	(Ezaki, 2015d)
Saccharopolyspora	Aerobe	(Kim and Goodfellow, 2015)
Salinispora	Aerobe	(Jensen et al., 2015)
Scardovia	Anaerobe	(Downes et al., 2011)
Schwartzia	Anaerobe	(Rainey, 2015)
Selenomonas	Anaerobe	(Shouche et al., 2015)
Sharpea	Anaerobe	(Morita et al., 2008)
Shuttleworthia	Anaerobe	(Wade and Downes, 2015)
Slackia	Anaerobe	(Wade and Dewhirst, 2015)
Sneathia	Anaerobe	(Eisenberg et al., 2018b)
Sphaerochaeta	Anaerobe	(Ritalahti et al., 2012)
Sphingomonas	Aerobe	(Yabuuchi and Kosako, 2015)

Staphylococcus	Facultative	(Schleifer and Bell, 2015)
Stenotrophomonas	Aerobe	(Palleroni, 2015b)
Streptococcus	Facultative	(Whiley and Hardie, 2015)
Streptomyces	Aerobe	(Kämpfer, 2015)
Sporobacterium	Anaerobe	(Mechichi and Patel, 2015)
Tannerella	Anaerobe	(Sakamoto et al., 2015)
Treponema	Anaerobe	(Norris et al., 2015)
Ureaplasma	Facultative	(Robertson et al., 2018)
Varibaculum	Facultative	(Hirakawa et al., 2015)
Variovorax	Aerobe	(Willems et al., 2015)
Veillonella	Anaerobe	(Carlier, 2015c)

Table E3. Correlation between ecological approaches show respiratory metabolism as key independent variables

	Aerobe	**Anaerobe**	**Facultative**	**Oral pH**	***Streptococcus***	***Prevotella***	***Neisseria***
Aerobe	1	-	-	-	-	-	-
Anaerobe	-0.2747	1	-	-	-	-	-
Facultative	-0.3762	-0.7295	1	-	-	-	-
Oral pH	0.2436	0.1163	-0.3788	1	-	-	-
Streptococcus	-0.211	-0.6937	0.851	-0.2137	1	-	-
Prevotella	-0.3173	0.8879	-0.6246	0.0545	-0.5992	1	-
Neisseria	0.7726	-0.0421	-0.5165	0.3617	-0.4017	-0.072	1

Table E4. Impact of antibacterial or immunosuppressive therapy on Pre-Tx dysbiosis.

	current antibacterials (n=7)	no antibacterials (n=15)	P value[a]	current immunosuppressive (n=11)	no immunosuppressive (n=11)	P value[a]
Richness[b]	311	285	0.45	306	280	0.37
Diversity[b]	3.88	3.58	0.33	3.80	3.55	0.27
PAM group 1[b]	6	13	1	8	11	0.21
PAM group 2	1	2		0	3	
PAM group 3	0	0		0	0	

[a] Richness and diversity were tested using Wilcoxon rank sum test, and for PAM group using Chi-square.

[b] Richness and diversity indicated by means, and PAM group by number in each category.

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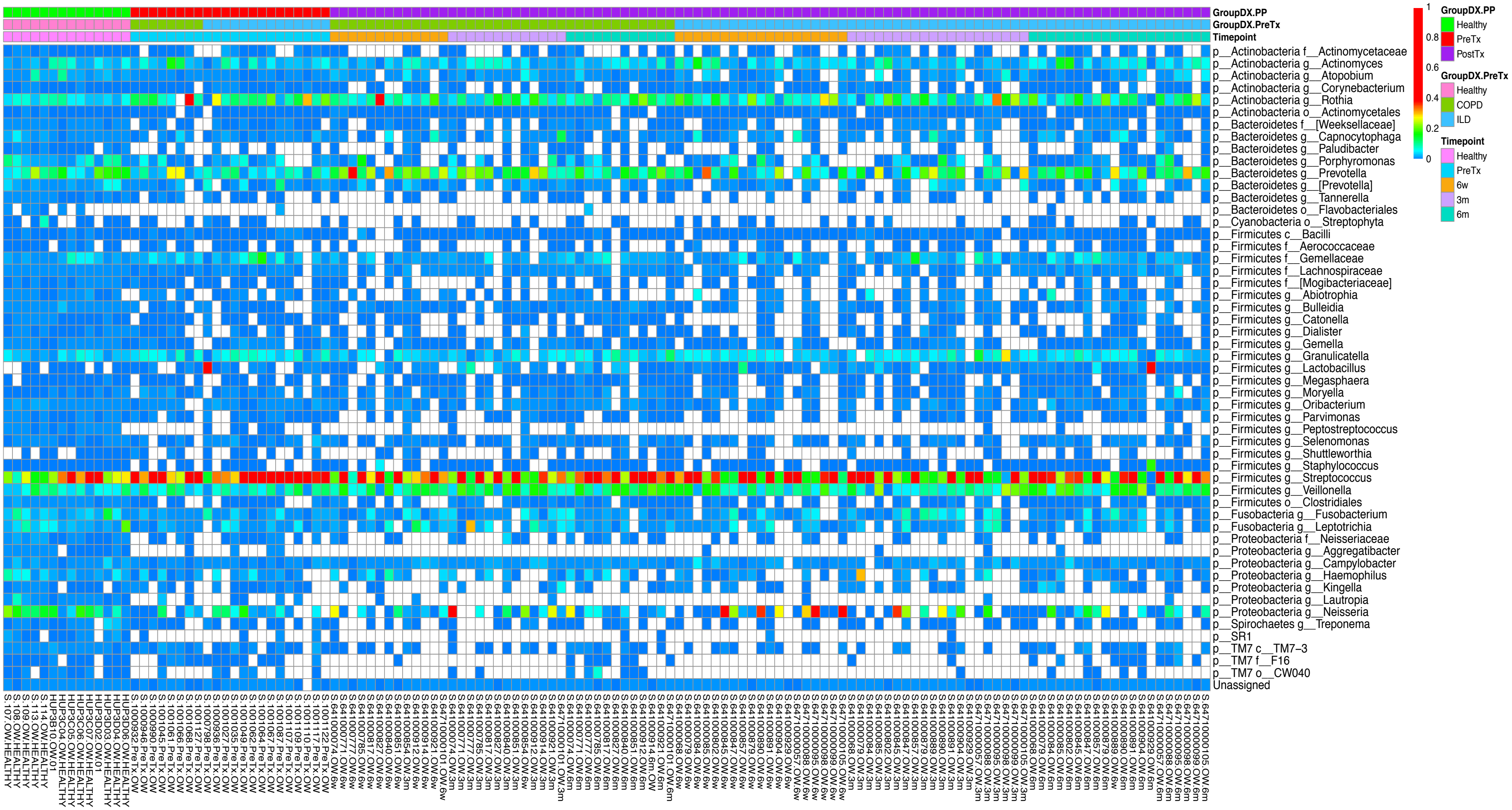

Figure E1. Bacterial composition based on 16S rRNA gene sequences in oral microbiome samples. The heat map shows oral samples in columns and bacterial taxa in rows. The first three rows indicate sample type. The first row shows disease groups (Healthy in green, Pre-Tx in red, and Post-Tx in purple). The second row corresponds to diagnosis groups (Healthy in pink, COPD in green, and ILD in blue), and third row by time point (Healthy in pink, Pre-Tx in blue, Post-Tx six weeks in orange, three months in purple and six months in turquoise). Relative abundance of indicated taxa within each sample is colored from blue to red, from 0 to 1 bacterial proportion per sample. White indicates absence of these taxa within the sample.

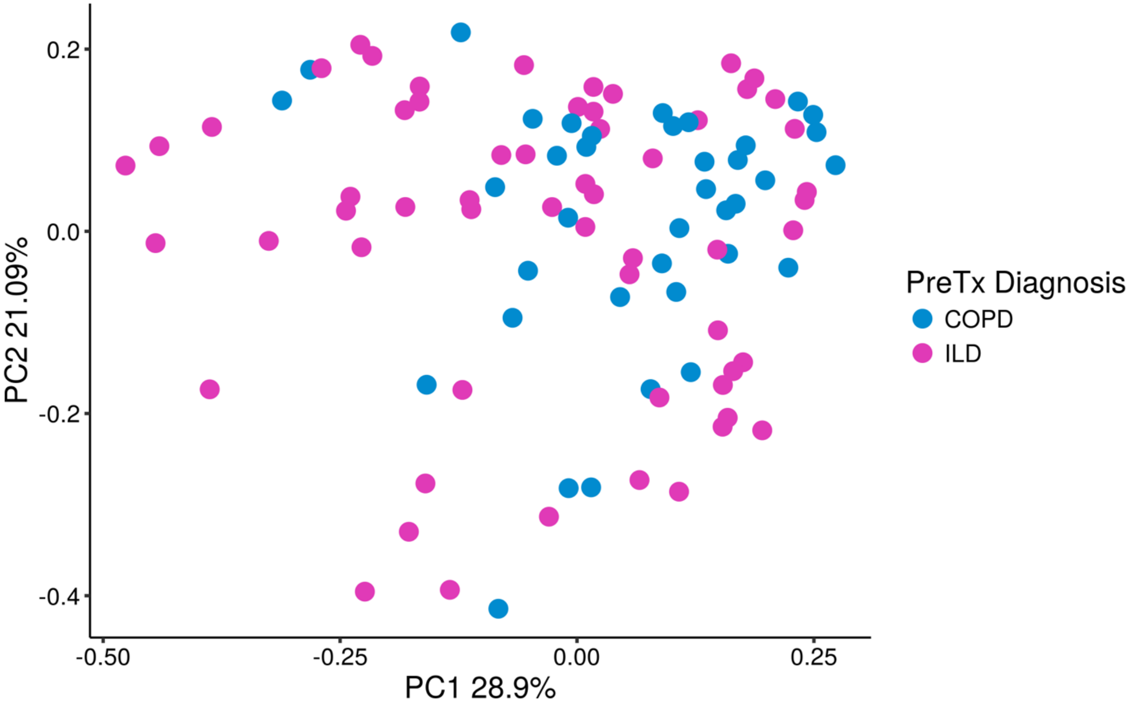

Figure E2. Distribution of the oropharyngeal microbiome among lung diagnosis groups. PCoA showing sample distribution based on lung disease group; Pre-Tx are based on disease and Post-Tx are based on disease indication for lung transplantations. Colors indicate COPD (red) or ILD (blue). No significant differences based on diagnosis were seen.

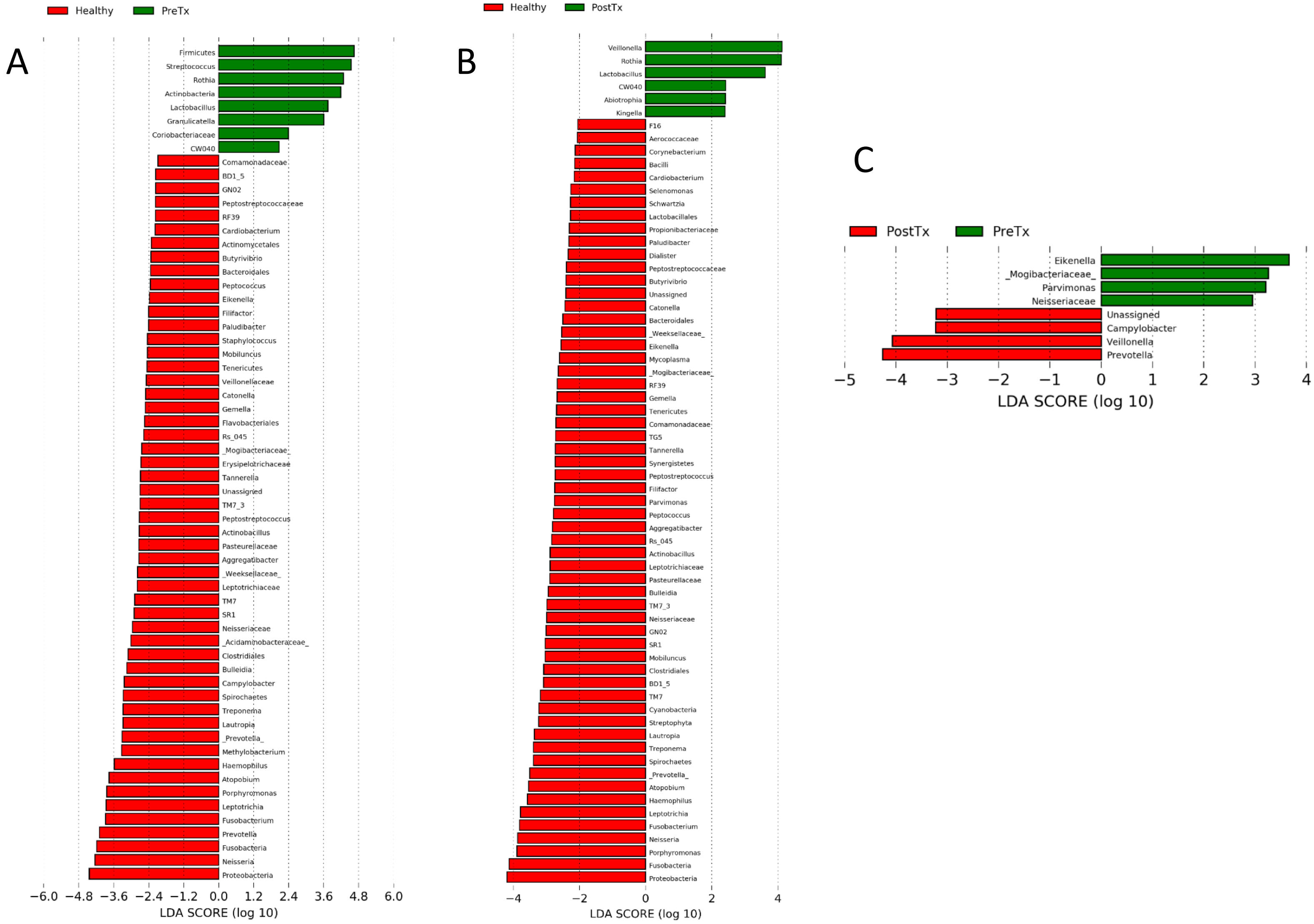

Figure E3. Linear Discriminant Analysis Effect Size (LEfSe) analysis of bacterial taxa associated with disease groups. Specific enriched taxa are indicated for (A) Pre-Tx compared to healthy communities; (B) Post-Tx compared to healthy communities, and; (C) Post-Tx compared to Pre-Tx communities. For analysis, Post-Tx time points were grouped together.

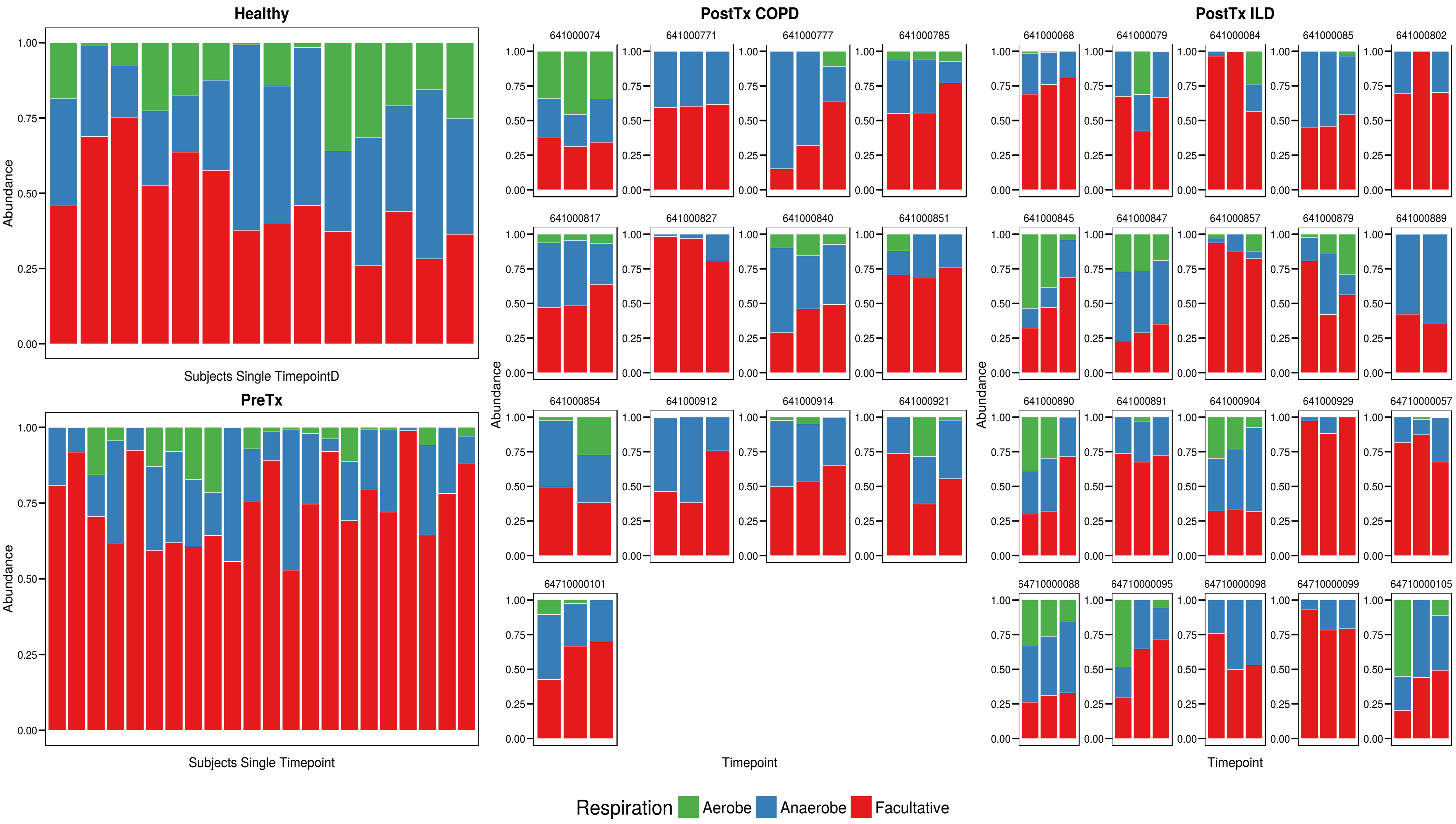

Figure E4. Bacterial respiration phenotype dynamics over time. Stacked bar graphs indicate the proportion of strict aerobic (green), obligate anaerobic (blue) and facultative anaerobic bacteria (red) within each sample. Samples are separated by disease group, diagnosis and time point. Single time points were evaluated for healthy and Pre-Tx groups; longitudinal samples are grouped by subject for the Post-Tx group (6 weeks, 3 months and 6 months).

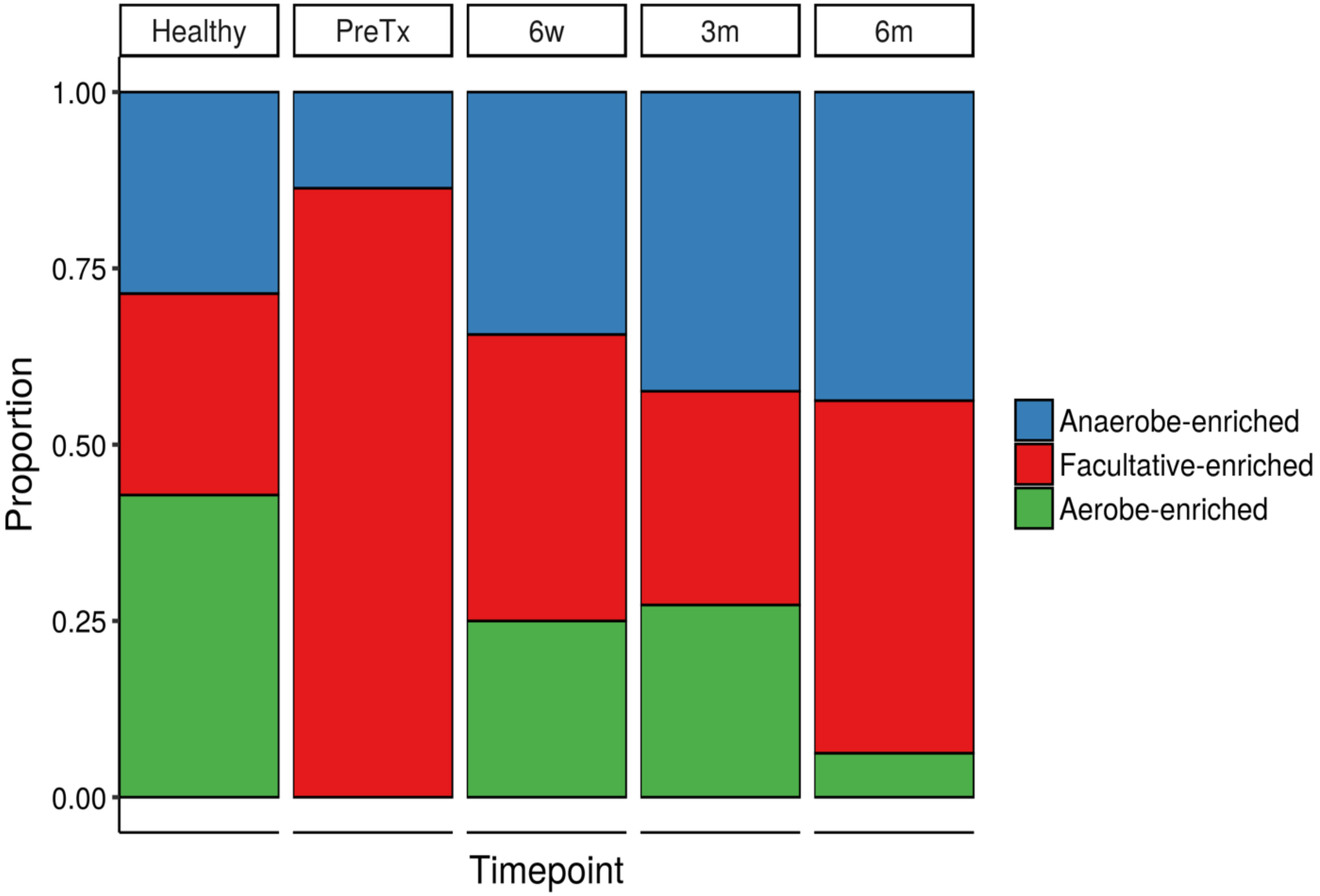

Figure E5. Bacterial respiration phenotype groups in oral samples. Partitioning around medoids (PAM) was applied to identify phenotype groups, with the number of clusters estimated by maximum average silhouette width. Clustering identified Anaerobe-enriched, Facultative-enriched, and Aerobe-enriched clusters, and the proportion of each is shown by timepoint.

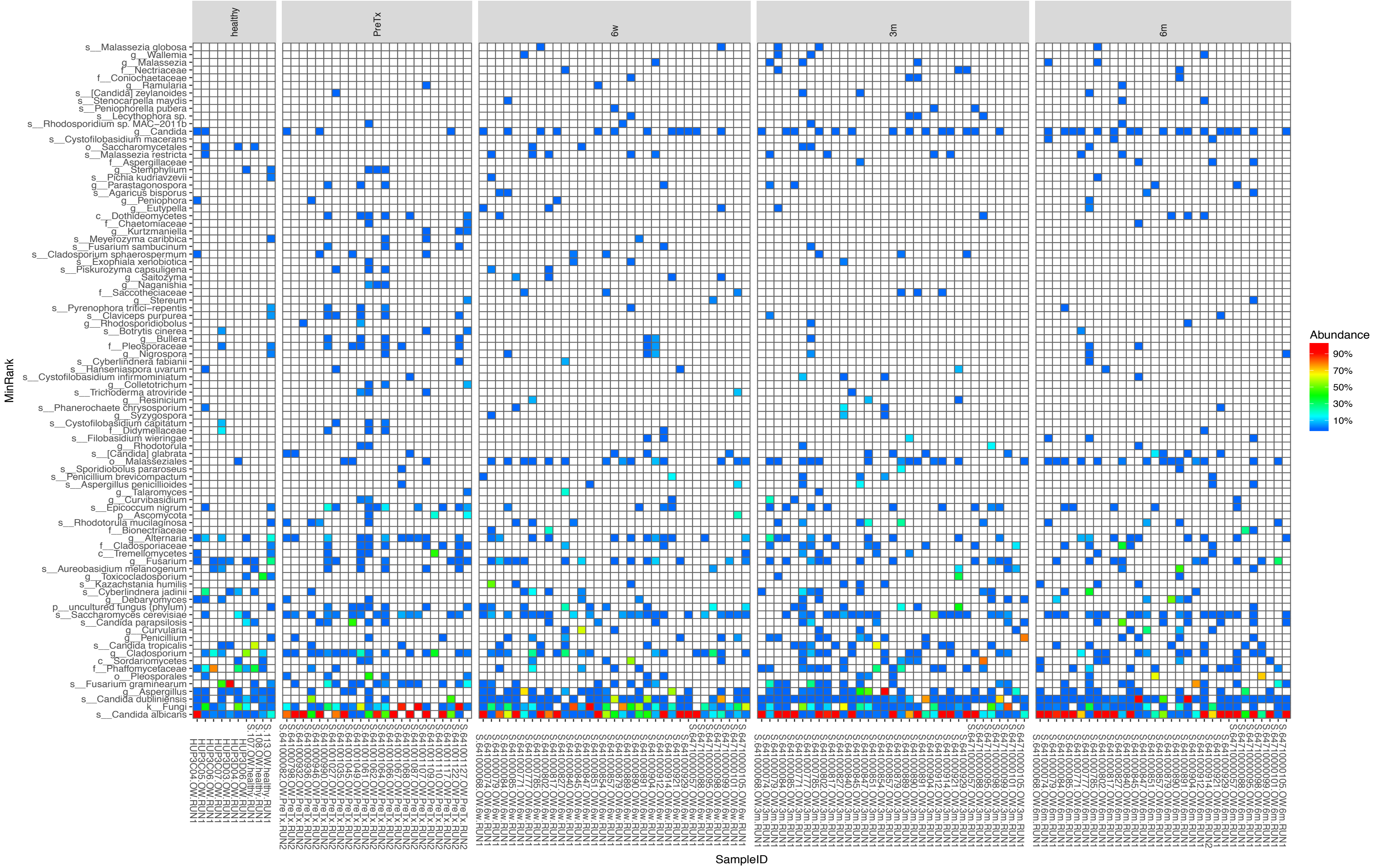

Figure E6. Fungal composition in oropharyngeal samples based on ITS rRNA gene sequences. The heat map shows relative abundances of the fungal taxa. Healthy and pre-transplantation have a single timepoint, longitudinal samples for post-transplant subjects are grouped by timepoint.

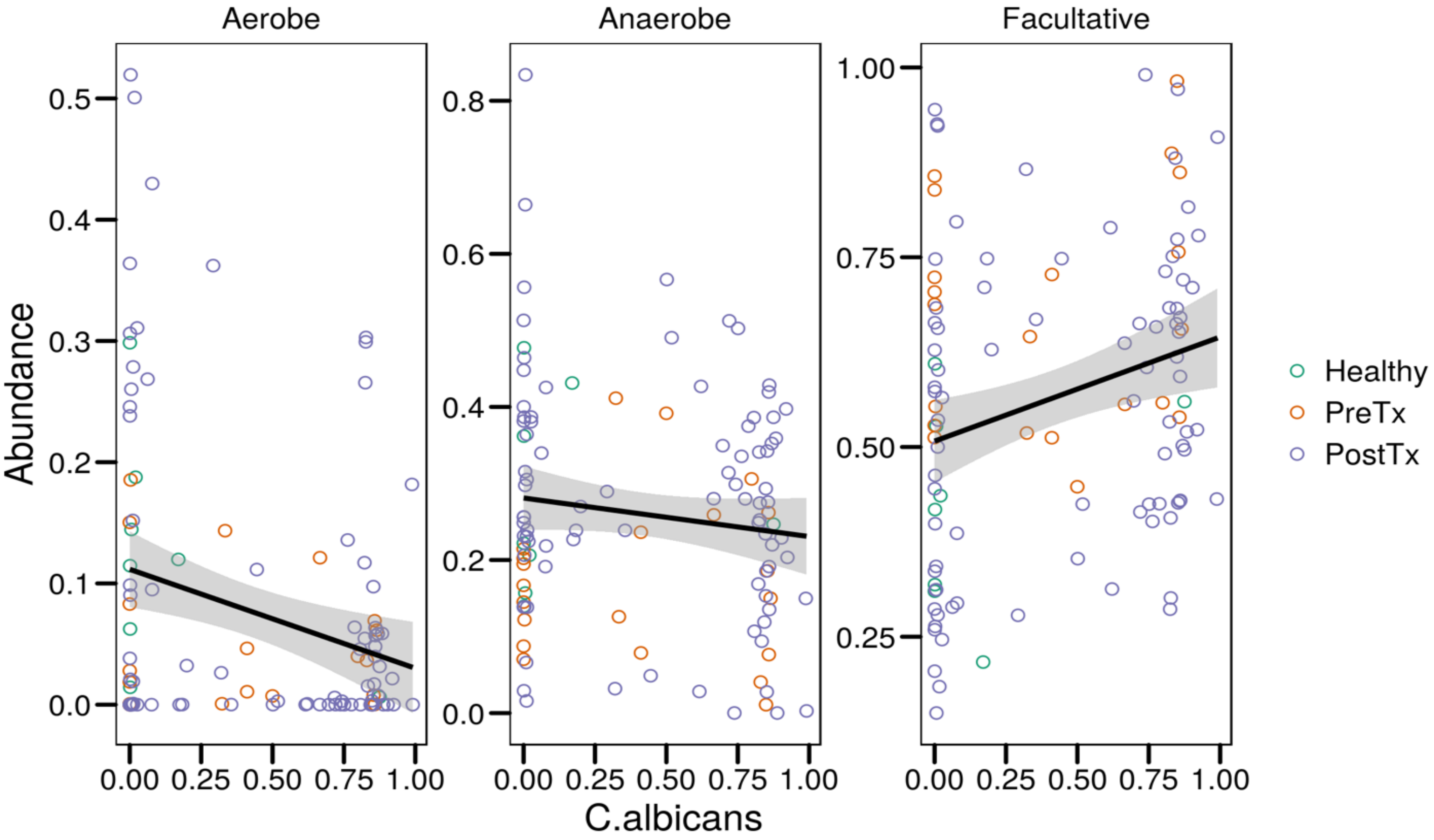

Figure E7. Correlation between Candida albicans and bacterial respiration phenotypes. Relative abundance of aerobe, anaerobe and facultative bacteria in each sample was compared to relative abundance of Candida albicans. C. albicans abundance is correlated inversely with aerobe and positively with facultative populations ($p<0.05$), but not with anaerobic bacteria ($p=0.17$). P-values were obtained from a linear model.