

# Supplemental Materials



## Periodontitis Bacteria in Gastric Cancer

Marcel A. de Leeuw & Manuel X. Duval, GeneCreek

### Contents

<b>Supplemental analyses</b>	<b>1</b>
microbial community types . . . . .	1
anatomic location . . . . .	2
MDS on UniFrac distances . . . . .	3
disease progress . . . . .	4
relevant species in GC . . . . .	5
comparison with CRC . . . . .	11
H. pylori proportion . . . . .	12
<b>Bibliography</b>	<b>14</b>

### List of Figures

S1	Goodness of fit of the DMM models at different k . . . . .	1
S2	Alpha diversity of community types . . . . .	2
S3	Interaction network between species relevant for gastric location . . . . .	3
S4	Multi-dimensional scaling of the disease status dataset SRP128749 . . . . .	4
S5	Multi-dimensional scaling of the disease progress data set . . . . .	4
S6	Shannon species diversity and disease progress (SRP070925) . . . . .	5
S7	Shannon species diversity and disease progress (ERP023334) . . . . .	5
S8	Interaction network between species relevant for disease progress (SRP070925) . . . . .	6
S9	Interaction network between species relevant for disease progress (ERP023334) . . . . .	6
S10	Discriminating species in CRC . . . . .	11
S11	Discriminating species in CRC . . . . .	12
S12	Helicobacter pylori proportion, DMMs . . . . .	13
S13	Helicobacter pylori proportion, SRP200169 + SRP070925 . . . . .	13
S14	Helicobacter pylori proportion, ERP023334 . . . . .	13
S15	Helicobacter pylori proportion, SRP128749 . . . . .	14
S16	Helicobacter pylori proportion, SRP172818 . . . . .	14

### List of Tables

S1	Gastric mucosa genera . . . . .	1
S2	RF classification of sampling location . . . . .	2
S3	prevalence differences between sample locations, SRP172818 . . . . .	7
S4	prevalence differences between sample locations, SRP128749 . . . . .	7
S5	prevalence differences between disease stages, SRP070925 . . . . .	8
S6	prevalence differences between disease stages, ERP023334 . . . . .	9
S7	prevalence differences between disease stages, ERP023334 . . . . .	10
S8	prevalence differences between CRC subtypes, SRP117763 . . . . .	11
S9	prevalence differences between CRC sample locations, SRP137015 . . . . .	11
S10	prevalence differences between CRC sample locations, SRP076561 . . . . .	12
S11	prevalence differences between CRC sample locations, ERP005534 . . . . .	12

### Supplemental analyses

#### microbial community types

Using Dirichlet Multinomial Mixtures on the combined relative abundances of the nine datasets (n=1,544) listed in table 1, we obtain an optimal goodness of fit at k=6 communities according to the Laplace evaluation, figure S1. The breakdown of samples from the various datasets along the community types is given in table 4.

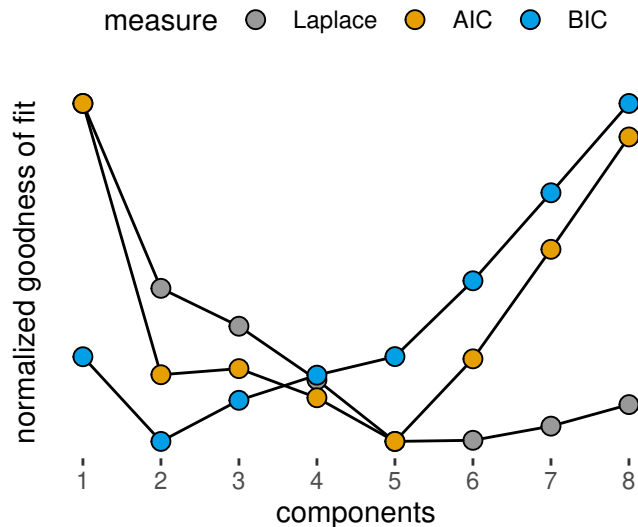


Figure S1: Goodness of fit of the DMM models at different k.

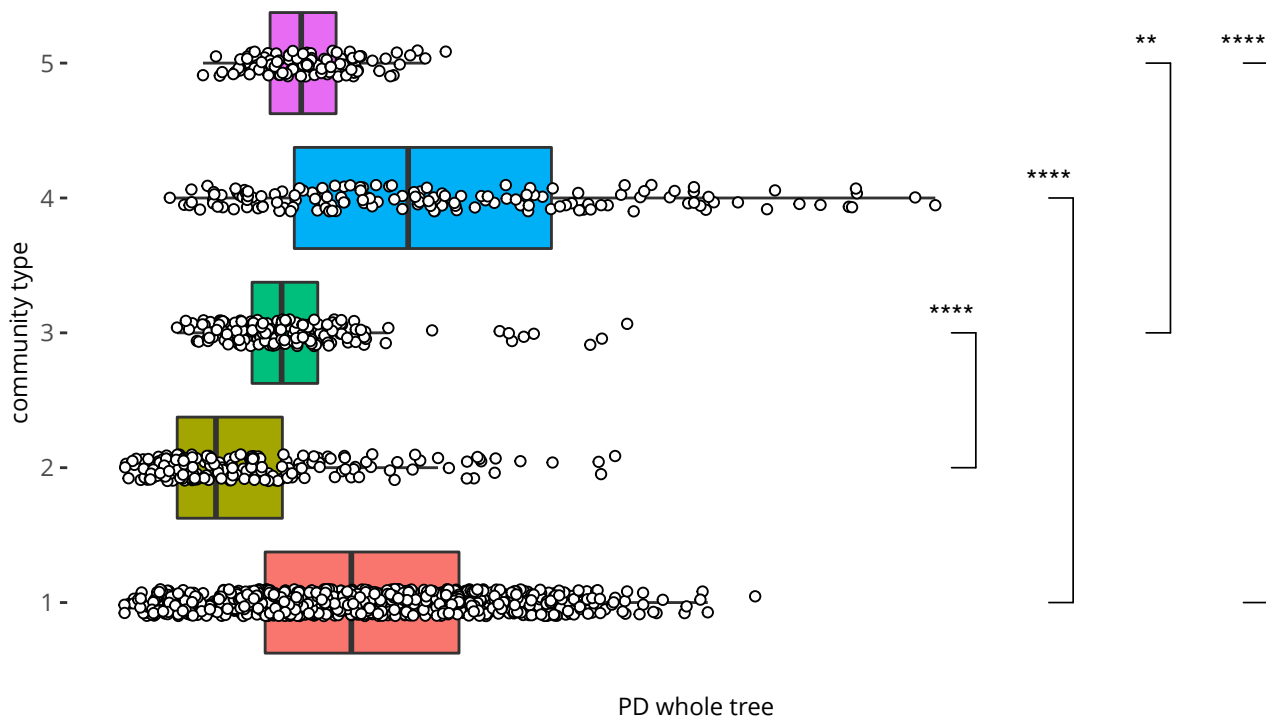
The top 100 discriminant species are distributed over 62 genera, with 18 genera encompassing more than one species, table S1.

Table S1: Gastric mucosa genera. Only genera with more than one species are listed.

genus	species
Prevotella	10
Streptococcus	9
Acinetobacter	4
Campylobacter	4
Porphyromonas	4
Arthrobacter	3
Fusobacterium	3
Leuconostoc	3
Methylobacterium	3
Sphingomonas	3
Veillonella	3
Actinomyces	2
Alloprevotella	2
Bacillus	2
Brevundimonas	2
Clostridium	2
Haemophilus	2
Lactococcus	2
Neisseria	2

Further indication that the DMMs are distinct in nature can be found in the projection of alpha diversity, using the phylogenetic diversity (whole tree), figure S2. The *Helicobacter pylori* dominated community type three has

the lowest diversity.



**Figure S2:** Alpha diversity of community types. Phylogenetic diversity (whole tree).

**anatomic location**

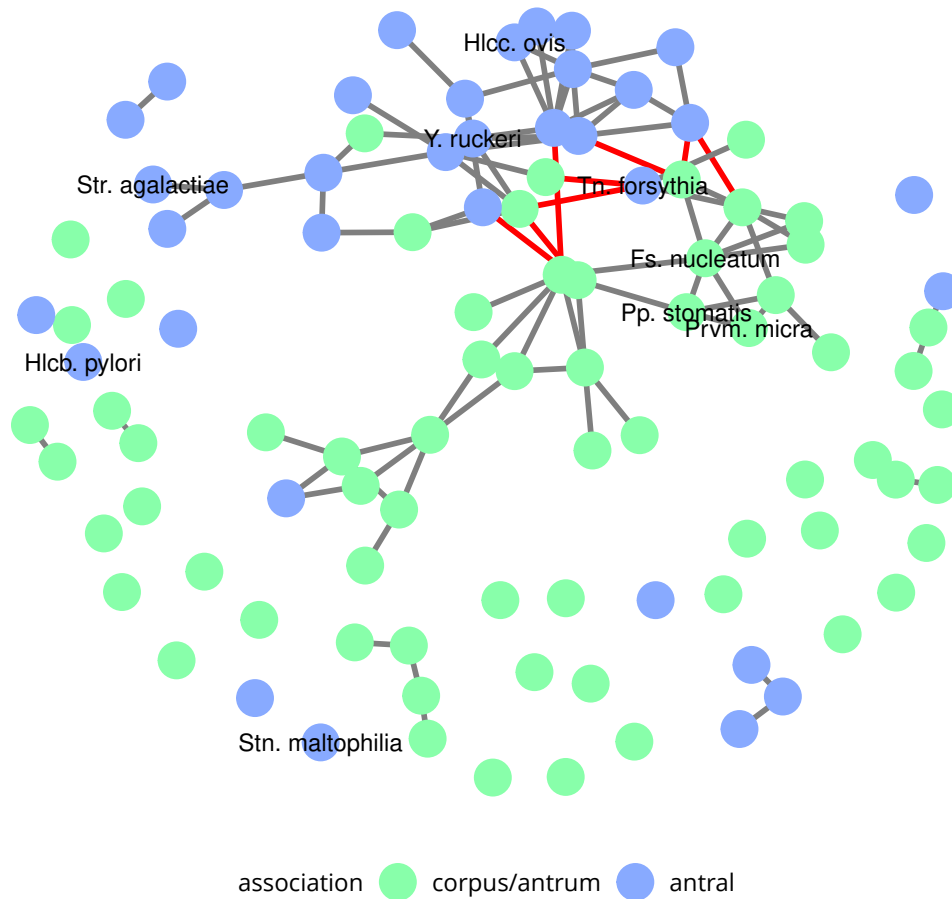
Data set SRP154244 presents samples from different gastric locations in patients with gastritis, intestinal metaplasia and gastric cancer. We investigate if microbial signatures differ per anatomic location by training an RF model on two thirds of the samples and evaluating the model on the remaining third. Table S3 suggests the antral is well differentiated from the antrum and body, but the latter two are not differentiated. Thus at first sight, gastric location could at least in part explain differences in community types.

**Table S2:** RF classification of sampling location. Predictions are in columns. Multiclass AUC:0.788

location	antral	antrum	body
antral	72	3	0
antrum	6	11	0
body	1	6	1

To shine further light on this matter, we group corpus and antrum samples together and retrain an RF model on the whole of the SRP154244 dataset, retrieve differentiating species and build a SPIEC-EASI network, figure S3. Although we find significant separation between the two locations, especially when considering the negative correlations (in red), the separation is not as strict as the separation between community types. So it does not seem we can explain the distribution of datasets over the community types by difference in anatomic location alone. Of note, we find three bacteria encountered in colorectal cancer, *Fusobacterium nucleatum*, *Parvimonas micra*, *Peptostreptococcus stomatis* in interaction and associated with the corpus/antrum. *Helicobacter pylori* is

more abundant in the antral and not in interaction with any other species.

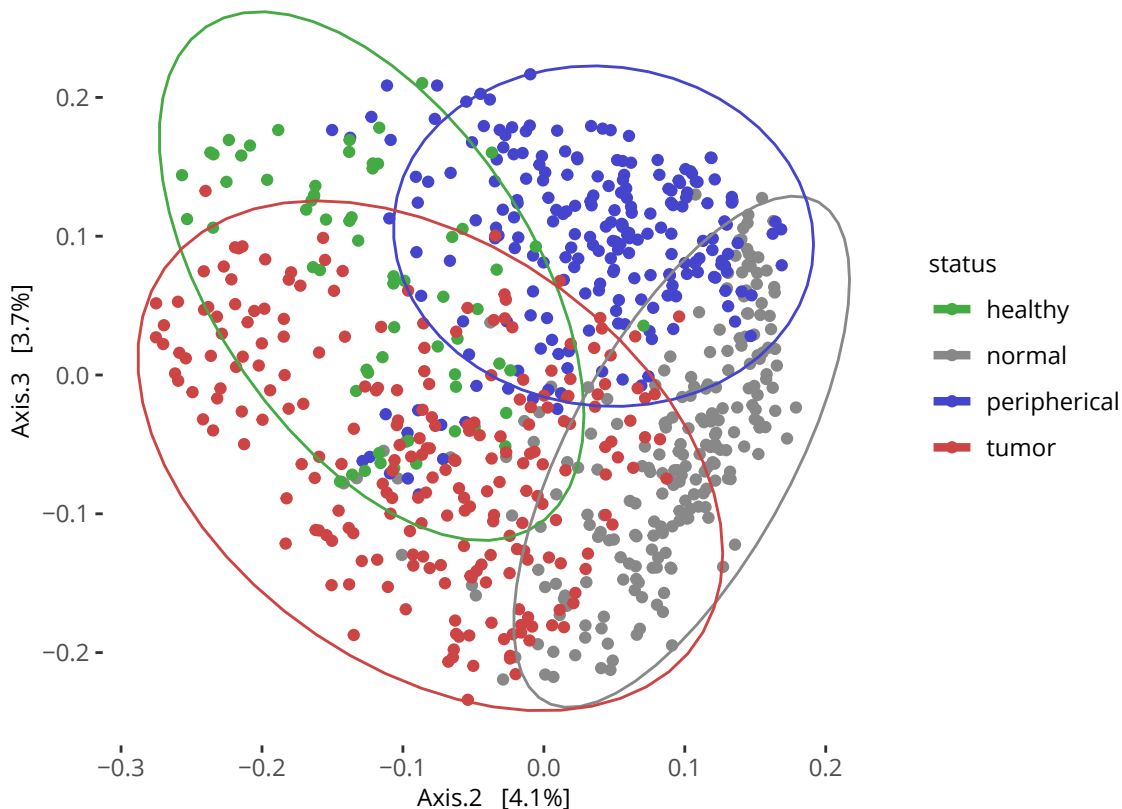


**Figure S3:** Interaction network between species relevant for gastric location. The top 100 species relevant for distinction between the two gastric locations are displayed. Opportunistic pathogens are labelled.

### MDS on UniFrac distances

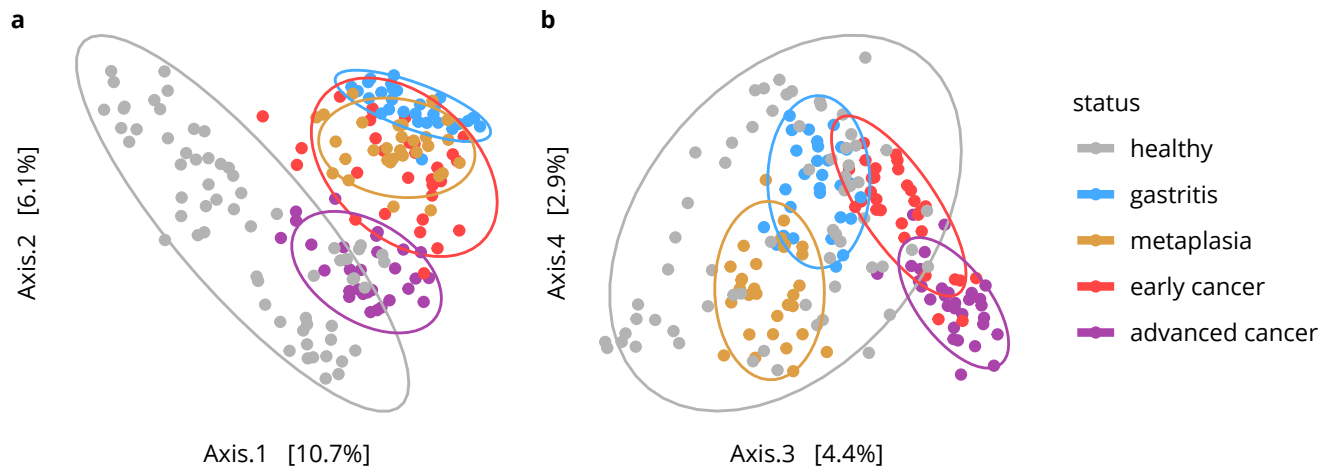
Using unweighted UniFrac distance on ASVs (amplicon sequence variants) we obtain better MDS separation of normal/peripheral/tumor samples than reported in [Liu et al. 2019], using the same dataset, whether without

(not shown) or with addition of samples from healthy donors, figure S4.



**Figure S4:** Multi-dimensional scaling of the disease status dataset SRP128749. Unweighted UniFrac of ASVs is used as the distance metric.

Data sets SRP070925 and SRP200169 combined also show interesting separation when performing multi-dimensional scaling on UniFrac distance, figure S5.

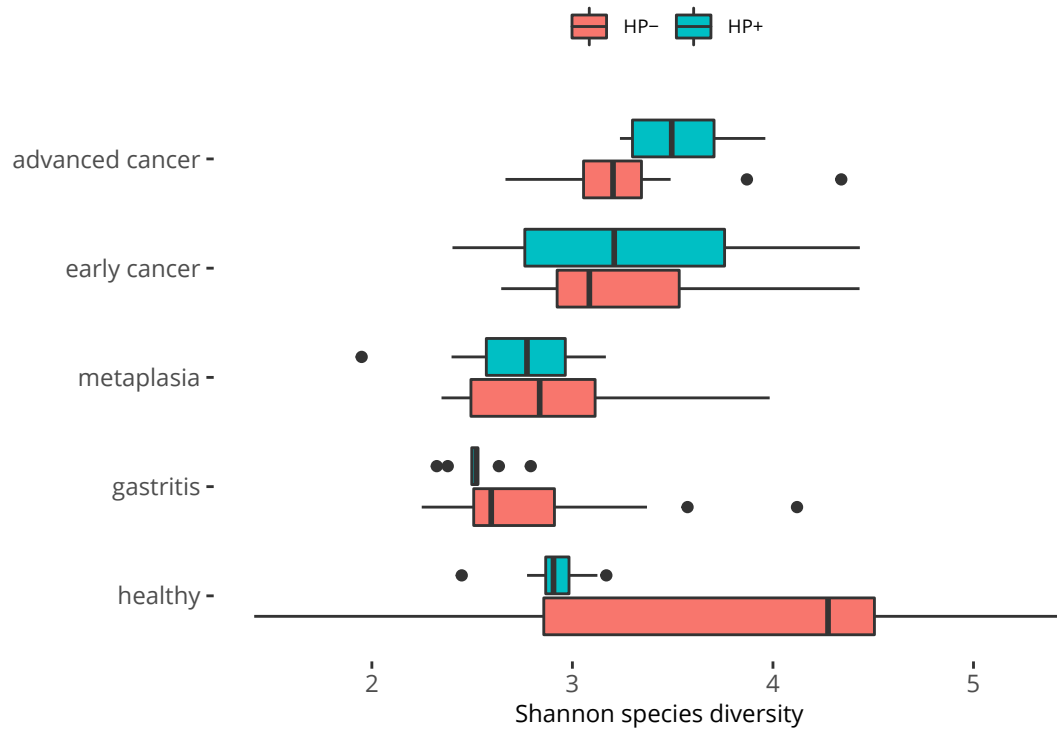


**Figure S5:** Multi-dimensional scaling of the disease progress data set. Unweighted UniFrac of ASVs is used as the distance metric.

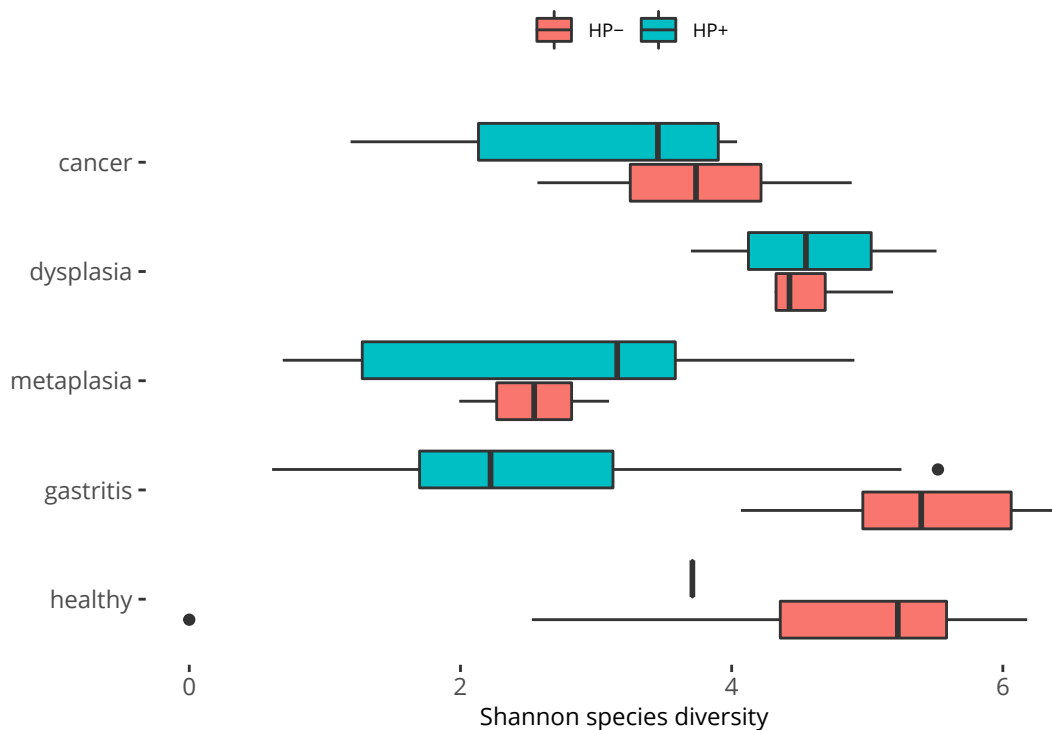
**disease progress**

The alpha diversity evolves along the disease progress path according to the Shannon criterion as applied to the SRP070925 dataset, figure S4. The gastritis is characterized by dysbiosis as compared to healthy tissue, with a

trend to reach normal diversity along the disease progress.



**Figure S6:** Shannon species diversity and disease progress (SRP070925). Helicobacter pylori positive (Hp+) and negative (Hp-) samples are distinguished.

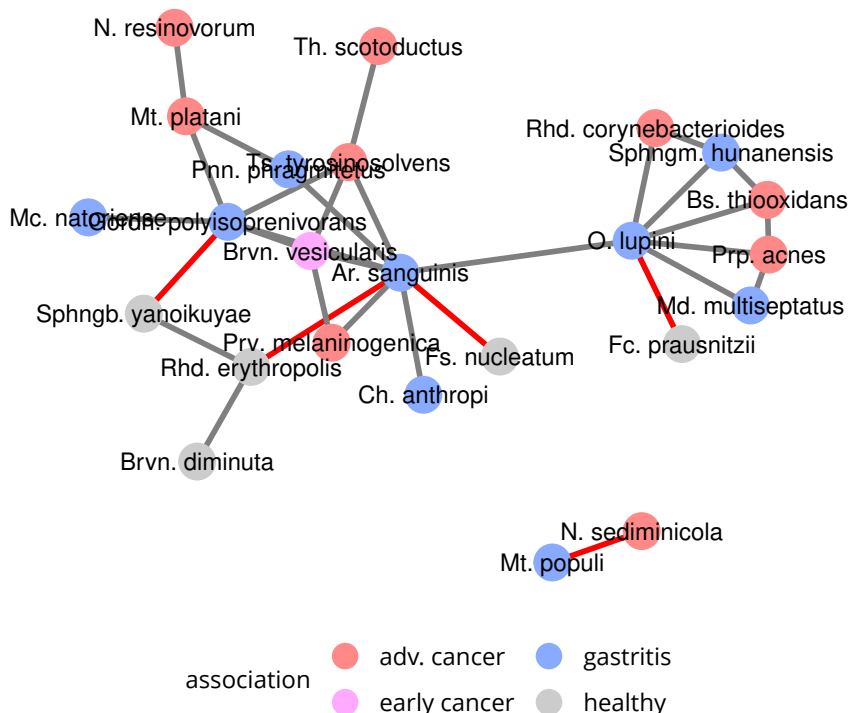


**Figure S7:** Shannon species diversity and disease progress (ERP023334). Helicobacter pylori positive (Hp+) and negative (Hp-) samples are distinguished.

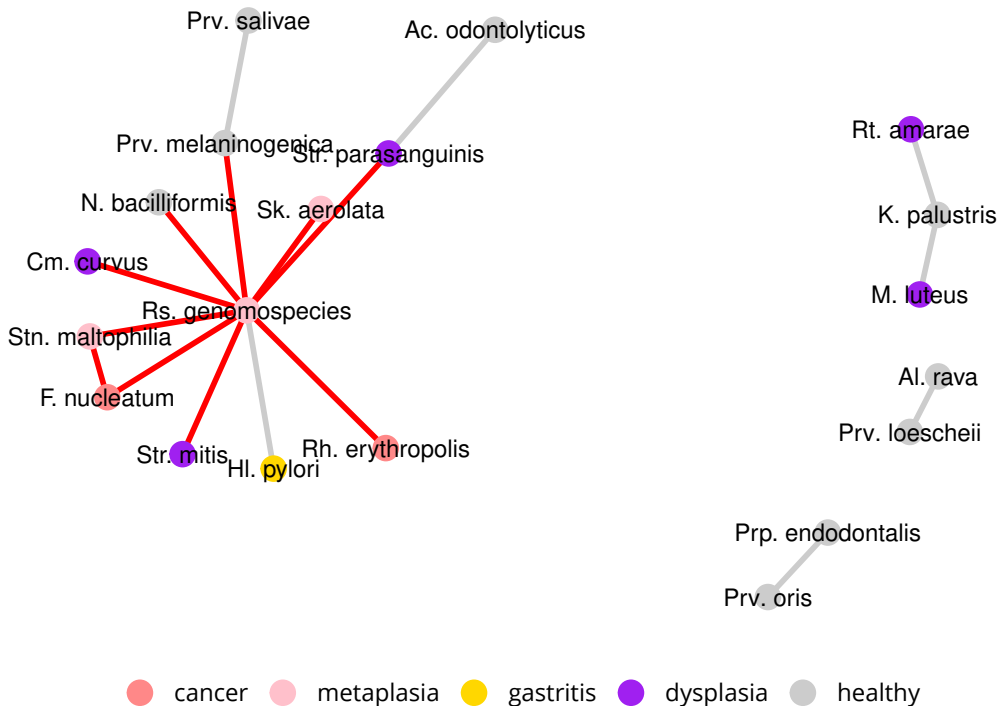
### relevant species in GC

We dispose of four datasets allowing for the association of species with tumor status, whether from a disease progress or tumor/normal status standpoint. We choose to process datasets individually because of possible regional differences and retrieve the top 50 differentiating species from the random forest models, which we

train on the datasets as a whole, so as to maximize performance. We provide sequence counts of these top 50 species to Spiec Easi for ecological network generation. We retain only connected nodes for display. Figure 3 provides the result for the two tumor/peripheral/normal datasets SRP128749 and SRP172818 alongside for comparison. Figure S5 below provides the same for the disease progress data set SRP070925, figure S6 for the disease progress data set ERP023334.



**Figure S8:** Interaction network between species relevant for disease progress (SRP070925). The top 50 species relevant for distinction between healthy the three disease stages are displayed. Only species with interactions are shown. Co-exclusion interactions are displayed in red.



**Figure S9:** Interaction network between species relevant for disease progress (ERP023334). The top 50 species relevant for distinction between healthy four disease stages are displayed. Only species with interactions are shown. Co-exclusion interactions are displayed in red.

We can further investigate species differences by inferring prevalence differences between disease states of

samples, using  $\chi^2$  testing, tables S4-S7 (separate MS Excel file GC\_suppl\_tables.xlsx).

**Table S3:** prevalence differences between sample locations, SRP172818.

species	association	pvalue		normal	peripheral	tumor	count
Fusobacterium mortiferum	normal	6.4e-05	***	9/57 (15.8%)	0/57 (0.0%)	0/59 (0.0%)	9
Streptomyces atroolivaceus	normal	3.2e-03	**	7/57 (12.3%)	1/57 (1.8%)	0/59 (0.0%)	8
Peptostreptococcus stomatis	peripheral,tumor	9.2e-03	**	15/57 (26.3%)	24/57 (42.1%)	32/59 (54.2%)	71
Corynebacterium tuberculostearicum	tumor	1.6e-04	***	2/57 (3.5%)	1/57 (1.8%)	13/59 (22.0%)	16
Propionibacterium acnes	tumor	3.5e-04	***	25/57 (43.9%)	22/57 (38.6%)	43/59 (72.9%)	90
Bifidobacterium dentium	tumor	5.6e-04	***	2/57 (3.5%)	3/57 (5.3%)	14/59 (23.7%)	19
Rudaeicoccus suwonensis	tumor	6.8e-04	***	3/57 (5.3%)	7/57 (12.3%)	18/59 (30.5%)	28
Deinococcus citri	tumor	1.6e-03	**	1/57 (1.8%)	0/57 (0.0%)	8/59 (13.6%)	9
Campylobacter rectus	tumor	1.6e-03	**	1/57 (1.8%)	0/57 (0.0%)	8/59 (13.6%)	9
Actinomyces odontolyticus	tumor	1.8e-03	**	1/57 (1.8%)	7/57 (12.3%)	14/59 (23.7%)	22
Rothia mucilaginosa	tumor	3.2e-03	**	10/57 (17.5%)	11/57 (19.3%)	25/59 (42.4%)	46
Fusobacterium nucleatum	tumor	5.7e-03	**	8/57 (14.0%)	12/57 (21.1%)	23/59 (39.0%)	43
Fusobacterium periodonticum	tumor	5.9e-03	**	1/57 (1.8%)	6/57 (10.5%)	12/59 (20.3%)	19
Alloprevotella tannerae	tumor	7.7e-03	**	1/57 (1.8%)	2/57 (3.5%)	9/59 (15.3%)	12
Prevotella melaninogenica	tumor	3.4e-02	*	2/57 (3.5%)	4/57 (7.0%)	10/59 (16.9%)	16
Helicobacter pylori	tumor	4.4e-02	*	47/57 (82.5%)	51/57 (89.5%)	57/59 (96.6%)	155
Parvimonas micra	tumor	7.3e-02		15/57 (26.3%)	14/57 (24.6%)	25/59 (42.4%)	54

**Table S4:** prevalence differences between sample locations, SRP128749.

species	association	pvalue		normal	peripheral	tumor	count
Fusobacterium mortiferum	normal	0.0e+00	***	25/225 (11.1%)	3/215 (1.4%)	1/229 (0.4%)	29
Bifidobacterium longum	normal	0.0e+00	***	42/225 (18.7%)	4/215 (1.9%)	18/229 (7.9%)	64
Clostridium cellulovorans	normal	1.3e-05	***	16/225 (7.1%)	2/215 (0.9%)	1/229 (0.4%)	19
Prevotella stercorea	normal	7.1e-05	***	22/225 (9.8%)	2/215 (0.9%)	9/229 (3.9%)	33
Nocardioides szechwanensis	normal	6.7e-04	***	9/225 (4.0%)	1/215 (0.5%)	0/229 (0.0%)	10
Roseburia inulinivorans	normal	9.1e-04	***	10/225 (4.4%)	0/215 (0.0%)	2/229 (0.9%)	12
Barnesiella intestinihominis	normal	9.3e-04	***	7/225 (3.1%)	0/215 (0.0%)	0/229 (0.0%)	7
Bacteroides uniformis	normal	2.4e-03	**	17/225 (7.6%)	2/215 (0.9%)	9/229 (3.9%)	28
Deinococcus aetherius	normal	2.5e-03	**	6/225 (2.7%)	0/215 (0.0%)	0/229 (0.0%)	6
Sulfurospirillum deleyianum	normal	2.5e-03	**	6/225 (2.7%)	0/215 (0.0%)	0/229 (0.0%)	6
Nitrospira japonica	normal	4.4e-03	**	11/225 (4.9%)	2/215 (0.9%)	2/229 (0.9%)	15
Parabacteroides merdae	normal	6.9e-03	**	5/225 (2.2%)	0/215 (0.0%)	0/229 (0.0%)	5
Ruminococcus bromii	normal,tumor	7.0e-07	***	57/225 (25.3%)	14/215 (6.5%)	42/229 (18.3%)	113
Faecalibacterium prausnitzii	normal,tumor	3.7e-06	***	127/225 (56.4%)	71/215 (33.0%)	111/229 (48.5%)	309
Arthrobacter oxydans	normal,tumor	1.2e-03	**	47/225 (20.9%)	28/215 (13.0%)	62/229 (27.1%)	137
Pyramidobacter pisolens	normal,tumor	3.4e-03	**	17/225 (7.6%)	2/215 (0.9%)	11/229 (4.8%)	30
Roseomonas gilardii	peripheral	2.2e-03	**	4/225 (1.8%)	20/215 (9.3%)	12/229 (5.2%)	36
Sphingomonas yabuuchiae	peripheral	7.2e-03	**	66/225 (29.3%)	94/215 (43.7%)	82/229 (35.8%)	242
Roseomonas stagni	peripheral	9.1e-03	**	4/225 (1.8%)	17/215 (7.9%)	10/229 (4.4%)	31
Helicobacter pylori	peripheral,tumor	4.8e-02	*	155/225 (68.9%)	169/215 (78.6%)	175/229 (76.4%)	499
Peptostreptococcus stomatis	tumor	0.0e+00	***	52/225 (23.1%)	60/215 (27.9%)	130/229 (56.8%)	242
Propionibacterium acnes	tumor	0.0e+00	***	82/225 (36.4%)	65/215 (30.2%)	140/229 (61.1%)	287
Parvimonas micra	tumor	3.0e-07	***	39/225 (17.3%)	40/215 (18.6%)	85/229 (37.1%)	164
Fusobacterium nucleatum	tumor	7.0e-07	***	34/225 (15.1%)	45/215 (20.9%)	82/229 (35.8%)	161
Campylobacter showae	tumor	6.4e-06	***	0/225 (0.0%)	1/215 (0.5%)	14/229 (6.1%)	15
Sphingomonas faeni	tumor	1.9e-05	***	29/225 (12.9%)	47/215 (21.9%)	71/229 (31.0%)	147
Catonella morbi	tumor	2.7e-05	***	7/225 (3.1%)	8/215 (3.7%)	29/229 (12.7%)	44
Thermus scotoductus	tumor	9.7e-05	***	63/225 (28.0%)	48/215 (22.3%)	93/229 (40.6%)	204
Corynebacterium tuberculostearicum	tumor	9.9e-05	***	10/225 (4.4%)	8/215 (3.7%)	30/229 (13.1%)	48
Leptotrichia wadei	tumor	2.3e-04	***	13/225 (5.8%)	20/215 (9.3%)	40/229 (17.5%)	73
Gardnerella vaginalis	tumor	7.6e-04	***	2/225 (0.9%)	1/215 (0.5%)	12/229 (5.2%)	15
Corynebacterium mucifaciens	tumor	1.7e-03	**	4/225 (1.8%)	10/215 (4.7%)	21/229 (9.2%)	35
Filifactor alois	tumor	4.3e-03	**	9/225 (4.0%)	13/215 (6.0%)	27/229 (11.8%)	49



**Table S5:** prevalence differences between disease stages, SRP070925.

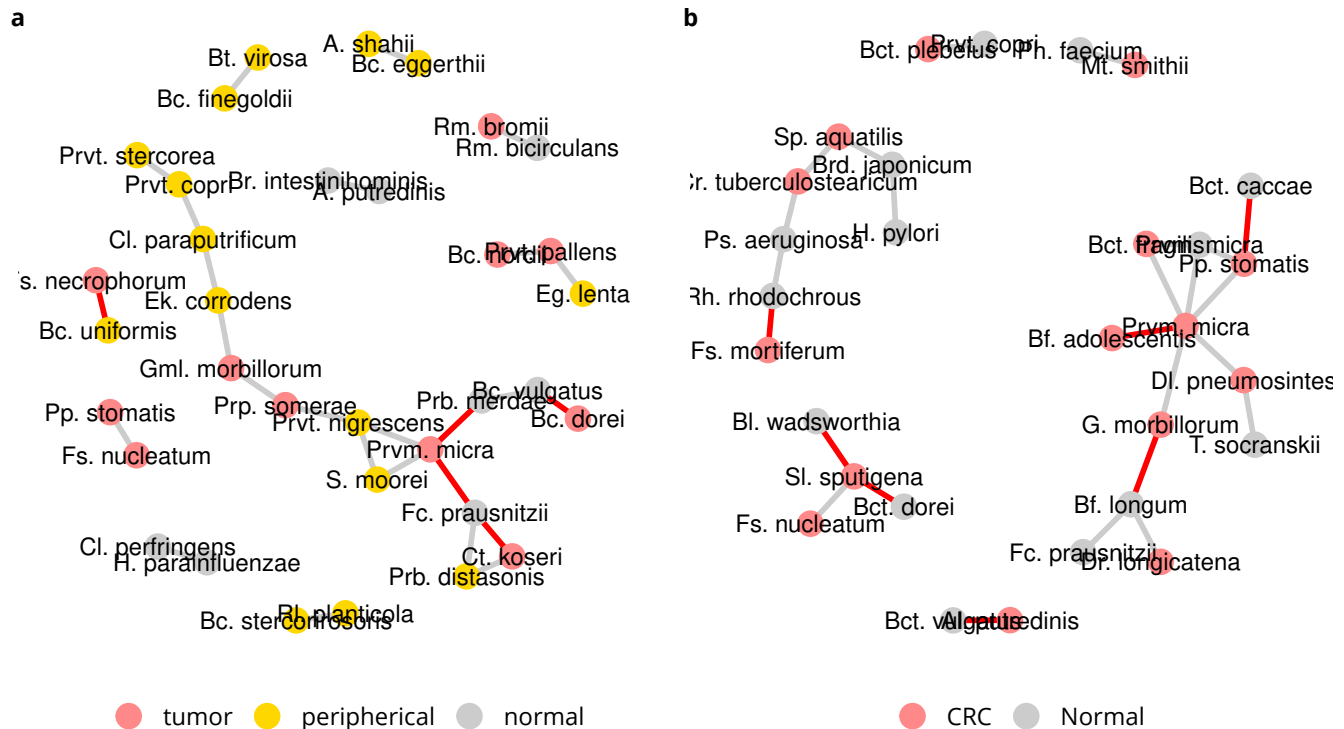
species	association	pvalue	advanced.cancer	early.cancer	gastritis	metaplasia	count
Peptostreptococcus stomatis	advanced cancer	2.6e-01	3/20 (15.0%)	0/20 (0.0%)	1/20 (5.0%)	1/20 (5.0%)	5
Novosphingobium sediminicola	early cancer,advanced cancer	0.0e+00 ***	15/20 (75.0%)	17/20 (85.0%)	0/20 (0.0%)	6/20 (30.0%)	38
Methylobacterium populi	gastritis	1.5e-03 **	7/20 (35.0%)	6/20 (30.0%)	17/20 (85.0%)	8/20 (40.0%)	38
Sphingobium amiense	gastritis	5.5e-03 **	0/20 (0.0%)	0/20 (0.0%)	4/20 (20.0%)	0/20 (0.0%)	4
Burkholderia cepacia	gastritis	6.7e-03 **	1/20 (5.0%)	0/20 (0.0%)	6/20 (30.0%)	1/20 (5.0%)	8
Sphingomonas hunanensis	gastritis	7.3e-03 **	1/20 (5.0%)	1/20 (5.0%)	8/20 (40.0%)	3/20 (15.0%)	13
Parvimonas micra	gastritis,advanced cancer	3.1e-01	3/20 (15.0%)	1/20 (5.0%)	2/20 (10.0%)	0/20 (0.0%)	6
Sphingomonas faeni	gastritis,early cancer	8.1e-03 **	2/20 (10.0%)	5/20 (25.0%)	10/20 (50.0%)	2/20 (10.0%)	19
Hyphomonas polymorpha	gastritis,metaplasia	8.0e-07 ***	4/20 (20.0%)	0/20 (0.0%)	16/20 (80.0%)	7/20 (35.0%)	27
Modestobacter multiseptatus	gastritis,metaplasia	1.9e-04 ***	0/20 (0.0%)	1/20 (5.0%)	10/20 (50.0%)	7/20 (35.0%)	18
Paenibacillus humicus	gastritis,metaplasia	5.1e-03 **	10/20 (50.0%)	12/20 (60.0%)	18/20 (90.0%)	18/20 (90.0%)	58
Geotoga petraea	gastritis,metaplasia	8.3e-03 **	3/20 (15.0%)	0/20 (0.0%)	7/20 (35.0%)	8/20 (40.0%)	18
Prevotella melaninogenica	gastritis,metaplasia	4.5e-01	3/20 (15.0%)	4/20 (20.0%)	6/20 (30.0%)	7/20 (35.0%)	20
Helicobacter pylori	gastritis,metaplasia	5.6e-01	13/20 (65.0%)	12/20 (60.0%)	14/20 (70.0%)	16/20 (80.0%)	55
Fusobacterium nucleatum	metaplasia,advanced cancer	1.6e-01	8/20 (40.0%)	4/20 (20.0%)	2/20 (10.0%)	5/20 (25.0%)	19

**Table S6:** prevalence differences between disease stages, ERP023334.

species	association	pvalue		cancer	dysplasia	gastritis	healthy	metaplasia	count
<i>Lactobacillus rhamnosus</i>	cancer	2.0e-03 **	2/10 (20.0%)	0/8 (0.0%)	0/44 (0.0%)	0/22 (0.0%)	0/9 (0.0%)		2
<i>Corynebacterium pseudodiphtheriticum</i>	dysplasia	5.0e-07 ***	0/10 (0.0%)	4/8 (50.0%)	0/44 (0.0%)	1/22 (4.5%)	0/9 (0.0%)		5
<i>Marmoricola aequoreus</i>	dysplasia	2.3e-04 ***	0/10 (0.0%)	2/8 (25.0%)	0/44 (0.0%)	0/22 (0.0%)	0/9 (0.0%)		2
<i>Parvimonas micra</i>	gastritis	6.9e-01	0/10 (0.0%)	0/8 (0.0%)	2/44 (4.5%)	0/22 (0.0%)	0/9 (0.0%)		2
<i>Prevotella fusca</i>	healthy	1.1e-05 ***	0/10 (0.0%)	0/8 (0.0%)	0/44 (0.0%)	8/22 (36.4%)	0/9 (0.0%)		8
<i>Haemophilus sputorum</i>	healthy	3.6e-04 ***	0/10 (0.0%)	0/8 (0.0%)	0/44 (0.0%)	6/22 (27.3%)	0/9 (0.0%)		6
<i>Prevotella veroralis</i>	healthy	4.8e-04 ***	1/10 (10.0%)	1/8 (12.5%)	6/44 (13.6%)	13/22 (59.1%)	1/9 (11.1%)		22
<i>Prevotella loescheii</i>	healthy	6.6e-04 ***	3/10 (30.0%)	1/8 (12.5%)	9/44 (20.5%)	15/22 (68.2%)	1/9 (11.1%)		29
<i>Prevotella dentalis</i>	healthy	1.2e-03 **	1/10 (10.0%)	1/8 (12.5%)	2/44 (4.5%)	9/22 (40.9%)	0/9 (0.0%)		13
<i>Treponema amylovorum</i>	healthy	1.6e-03 **	0/10 (0.0%)	0/8 (0.0%)	3/44 (6.8%)	8/22 (36.4%)	0/9 (0.0%)		11
<i>Prevotella oulorum</i>	healthy	1.6e-03 **	3/10 (30.0%)	3/8 (37.5%)	12/44 (27.3%)	17/22 (77.3%)	2/9 (22.2%)		37
<i>Tannerella forsythia</i>	healthy	3.8e-03 **	3/10 (30.0%)	3/8 (37.5%)	13/44 (29.5%)	16/22 (72.7%)	1/9 (11.1%)		36
<i>Prevotella pallens</i>	healthy	4.2e-03 **	3/10 (30.0%)	3/8 (37.5%)	17/44 (38.6%)	17/22 (77.3%)	1/9 (11.1%)		41
<i>Treponema denticola</i>	healthy	4.6e-03 **	2/10 (20.0%)	1/8 (12.5%)	6/44 (13.6%)	11/22 (50.0%)	0/9 (0.0%)		20
<i>Propionibacterium acnes</i>	healthy	5.1e-02	1/10 (10.0%)	2/8 (25.0%)	8/44 (18.2%)	11/22 (50.0%)	2/9 (22.2%)		24
<i>Porphyromonas endodontalis</i>	healthy,cancer	1.5e-05 ***	4/10 (40.0%)	2/8 (25.0%)	9/44 (20.5%)	19/22 (86.4%)	3/9 (33.3%)		37
<i>Alloprevotella rava</i>	healthy,cancer	3.7e-05 ***	4/10 (40.0%)	2/8 (25.0%)	8/44 (18.2%)	17/22 (77.3%)	1/9 (11.1%)		32
<i>Solobacterium moorei</i>	healthy,cancer	6.7e-04 ***	3/10 (30.0%)	2/8 (25.0%)	6/44 (13.6%)	13/22 (59.1%)	0/9 (0.0%)		24
<i>Neisseria elongata</i>	healthy,dysplasia	2.1e-05 ***	1/10 (10.0%)	3/8 (37.5%)	9/44 (20.5%)	17/22 (77.3%)	1/9 (11.1%)		31
<i>Haemophilus parainfluenzae</i>	healthy,dysplasia	5.8e-05 ***	4/10 (40.0%)	8/8 (100.0%)	17/44 (38.6%)	19/22 (86.4%)	2/9 (22.2%)		50
<i>Prevotella oris</i>	healthy,dysplasia	1.2e-04 ***	2/10 (20.0%)	5/8 (62.5%)	12/44 (27.3%)	18/22 (81.8%)	2/9 (22.2%)		39
<i>Selenomonas diana</i>	healthy,dysplasia	1.5e-04 ***	3/10 (30.0%)	4/8 (50.0%)	12/44 (27.3%)	18/22 (81.8%)	1/9 (11.1%)		38
<i>Lautropia mirabilis</i>	healthy,dysplasia	2.0e-04 ***	2/10 (20.0%)	5/8 (62.5%)	10/44 (22.7%)	16/22 (72.7%)	1/9 (11.1%)		34
<i>Actinomyces odontolyticus</i>	healthy,dysplasia	6.9e-04 ***	5/10 (50.0%)	8/8 (100.0%)	18/44 (40.9%)	19/22 (86.4%)	4/9 (44.4%)		54
<i>Bradyrhizobium elkanii</i>	healthy,dysplasia	7.8e-04 ***	0/10 (0.0%)	3/8 (37.5%)	0/44 (0.0%)	3/22 (13.6%)	0/9 (0.0%)		6
<i>Capnocytophaga gingivalis</i>	healthy,dysplasia	8.3e-04 ***	2/10 (20.0%)	4/8 (50.0%)	8/44 (18.2%)	15/22 (68.2%)	2/9 (22.2%)		31
<i>Prevotella intermedia</i>	healthy,dysplasia	1.0e-03 **	1/10 (10.0%)	2/8 (25.0%)	6/44 (13.6%)	12/22 (54.5%)	0/9 (0.0%)		21
<i>Alloprevotella tanneriae</i>	healthy,dysplasia	1.6e-03 **	2/10 (20.0%)	4/8 (50.0%)	16/44 (36.4%)	17/22 (77.3%)	1/9 (11.1%)		40
<i>Campylobacter curvus</i>	healthy,dysplasia	1.9e-03 **	4/10 (40.0%)	6/8 (75.0%)	17/44 (38.6%)	19/22 (86.4%)	3/9 (33.3%)		49
<i>Actinomyces graevenitzi</i>	healthy,dysplasia	1.9e-03 **	1/10 (10.0%)	5/8 (62.5%)	12/44 (27.3%)	15/22 (68.2%)	2/9 (22.2%)		35
<i>Prevotella salivae</i>	healthy,dysplasia	1.9e-03 **	5/10 (50.0%)	7/8 (87.5%)	21/44 (47.7%)	19/22 (86.4%)	2/9 (22.2%)		54
<i>Aggregatibacter segnis</i>	healthy,dysplasia	2.0e-03 **	0/10 (0.0%)	2/8 (25.0%)	7/44 (15.9%)	12/22 (54.5%)	1/9 (11.1%)		22
<i>Veillonella atypica</i>	healthy,dysplasia	2.9e-03 **	4/10 (40.0%)	7/8 (87.5%)	16/44 (36.4%)	15/22 (68.2%)	1/9 (11.1%)		43
<i>Porphyromonas catoniae</i>	healthy,dysplasia	3.1e-03 **	2/10 (20.0%)	5/8 (62.5%)	16/44 (36.4%)	17/22 (77.3%)	2/9 (22.2%)		42
<i>Streptococcus salivarius</i>	healthy,dysplasia	3.7e-03 **	3/10 (30.0%)	7/8 (87.5%)	20/44 (45.5%)	17/22 (77.3%)	2/9 (22.2%)		49
<i>Veillonella parvula</i>	healthy,dysplasia	5.0e-03 **	3/10 (30.0%)	6/8 (75.0%)	15/44 (34.1%)	15/22 (68.2%)	1/9 (11.1%)		40
<i>Streptococcus parasanguinis</i>	healthy,dysplasia,cancer	4.1e-05 ***	7/10 (70.0%)	8/8 (100.0%)	16/44 (36.4%)	20/22 (90.9%)	4/9 (44.4%)		55
<i>Neisseria bacilliformis</i>	healthy,dysplasia,cancer	1.5e-04 ***	2/10 (20.0%)	2/8 (25.0%)	2/44 (4.5%)	11/22 (50.0%)	0/9 (0.0%)		17
<i>Atopobium parvulum</i>	healthy,dysplasia,cancer	4.5e-03 **	3/10 (30.0%)	3/8 (37.5%)	7/44 (15.9%)	12/22 (54.5%)	0/9 (0.0%)		25
<i>Fusobacterium nucleatum</i>	healthy,dysplasia,cancer	7.5e-03 **	8/10 (80.0%)	7/8 (87.5%)	25/44 (56.8%)	18/22 (81.8%)	2/9 (22.2%)		60
<i>Sphingomonas paucimobilis</i>	metaplasia	7.6e-04 ***	0/10 (0.0%)	0/8 (0.0%)	0/44 (0.0%)	0/22 (0.0%)	2/9 (22.2%)		2

**Table S7:** prevalence differences between disease stages, ERP023334.

species	association	pvalue		functional.dyspepsia	gastric.cancer	gastric.ulcer	count
Lachnoanaerobaculum umeaense	functional dyspepsia	7.0e-03	**	2/6 (33.3%)	0/15 (0.0%)	0/13 (0.0%)	2
Aquabacterium parvum	functional dyspepsia	7.0e-03	**	2/6 (33.3%)	0/15 (0.0%)	0/13 (0.0%)	2
Methylobacterium radiotolerans	functional dyspepsia,gastric ulcer	1.3e-04	***	6/6 (100.0%)	5/15 (33.3%)	13/13 (100.0%)	24
Lactococcus lactis	gastric cancer	5.2e-04	***	2/6 (33.3%)	12/15 (80.0%)	1/13 (7.7%)	15
Comamonas testosteroni	gastric cancer	9.9e-03	**	0/6 (0.0%)	6/15 (40.0%)	0/13 (0.0%)	6
Peptostreptococcus stomatis	gastric cancer	2.6e-01		0/6 (0.0%)	2/15 (13.3%)	0/13 (0.0%)	2
Parvimonas micra	gastric cancer	5.2e-01		0/6 (0.0%)	1/15 (6.7%)	0/13 (0.0%)	1
Fusobacterium nucleatum	gastric cancer,gastric ulcer	6.4e-01		1/6 (16.7%)	5/15 (33.3%)	5/13 (38.5%)	11



**Figure S10:** Discriminating species in CRC. Data sets a) SRP137015 and b) SRP076561. Only species with interactions are displayed. Location associations are based on maximum mean relative abundance. Co-exclusion is indicated in red.

### comparison with CRC

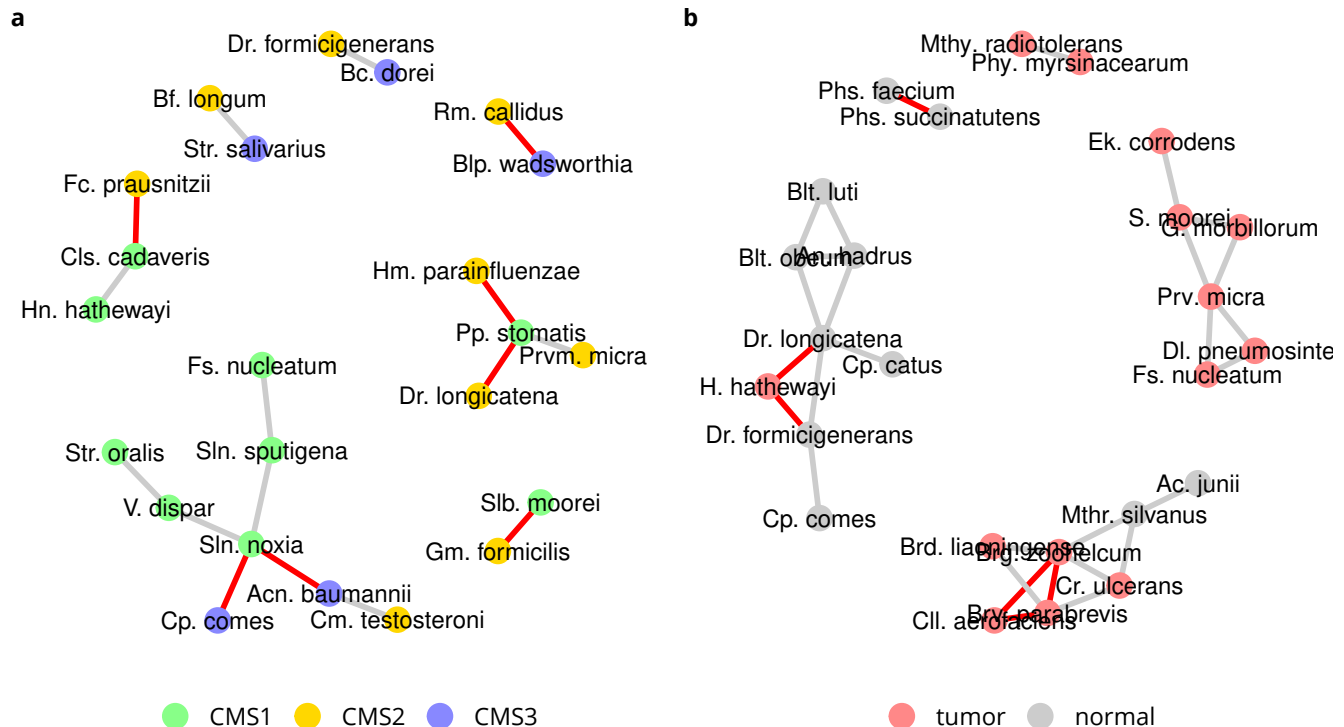
We test two CRC data sets for presence and interactions of *F. nucleatum*, *P. micra* and *P. stomatis*. Data set SRP117763 (n=34, tumor-only) was published by [Purcell et al. 2017] and data set SRP137015 (n=211, tumor/peripheral/normal) by [Hale et al. 2018b;a]. We find *F. nucleatum* in interaction with *P. stomatis* in SRP137015 and *P. micra* in interaction with *P. stomatis* in SRP117763, figure S@ref(fig:CRcgnet). Prevalence of *F. nucleatum* is over 70% in tumor samples in SRP117763, table S8 and at 48% in SRP137015, table S9.

**Table S8:** prevalence differences between CRC subtypes, SRP117763.

species	association	pvalue	CMS1	CMS2	CMS3	count
<i>Clostridium cadaveris</i>	CMS1	5.1e-03 **	4/6 (66.7%)	2/13 (15.4%)	0/10 (0.0%)	6
<i>Parvimonas micra</i>	CMS1,CMS2	4.0e-02 *	3/6 (50.0%)	8/13 (61.5%)	1/10 (10.0%)	12
<i>Peptostreptococcus stomatis</i>	CMS1,CMS2	1.8e-01	2/6 (33.3%)	6/13 (46.2%)	1/10 (10.0%)	9
<i>Prevotella melaninogenica</i>	CMS1,CMS2	4.4e-01	1/6 (16.7%)	1/13 (7.7%)	0/10 (0.0%)	2
<i>Fusobacterium nucleatum</i>	CMS1,CMS2	8.3e-01	5/6 (83.3%)	10/13 (76.9%)	7/10 (70.0%)	22

**Table S9:** prevalence differences between CRC sample locations, SRP137015.

species	association	pvalue	normal	peripheral	tumor	count
<i>Prevotella melaninogenica</i>	normal	5.9e-01	1/103 (1.0%)	0/46 (0.0%)	0/62 (0.0%)	1
<i>Bacteroides vulgatus</i>	normal,peripheral	1.3e-03 **	80/103 (77.7%)	38/46 (82.6%)	34/62 (54.8%)	152
<i>Peptostreptococcus stomatis</i>	peripheral,tumor	1.7e-01	12/103 (11.7%)	8/46 (17.4%)	14/62 (22.6%)	34
<i>Fusobacterium nucleatum</i>	tumor	3.4e-04 ***	20/103 (19.4%)	12/46 (26.1%)	30/62 (48.4%)	62
<i>Campylobacter gracilis</i>	tumor	1.5e-03 **	1/103 (1.0%)	1/46 (2.2%)	8/62 (12.9%)	10
<i>Parvimonas micra</i>	tumor	2.4e-03 **	5/103 (4.9%)	5/46 (10.9%)	14/62 (22.6%)	24



**Figure S11:** Discriminating species in CRC. Data sets a) SRP117763 and b) ERP005534 Only species with interactions are displayed. Location associations are based on maximum mean relative abundance. Co-exclusion is indicated in red.

**Table S10:** prevalence differences between CRC sample locations, SRP076561.

species	association	pvalue	CRC	Normal	count
Fusobacterium nucleatum	tumor	0.17	19/26 (73.1%)	12/24 (50.0%)	31
Prevotella melaninogenica	tumor	1.00	1/26 (3.8%)	0/24 (0.0%)	1
Propionibacterium acnes	normal	0.17	8/26 (30.8%)	13/24 (54.2%)	21
Parvimonas micra	normal	0.72	15/26 (57.7%)	16/24 (66.7%)	31
Helicobacter pylori	normal	0.74	14/26 (53.8%)	15/24 (62.5%)	29
Peptostreptococcus stomatis	normal	1.00	16/26 (61.5%)	15/24 (62.5%)	31

**Table S11:** prevalence differences between CRC sample locations, ERP005534.

species	association	pvalue	normal	tumor	count
Parvimonas micra		1.00	33/48 (68.8%)	33/48 (68.8%)	66
Prevotella melaninogenica	normal	0.47	2/48 (4.2%)	0/48 (0.0%)	2
Fusobacterium nucleatum	tumor	0.11	31/48 (64.6%)	39/48 (81.2%)	70
Peptostreptococcus stomatis	tumor	0.68	22/48 (45.8%)	25/48 (52.1%)	47

### H. pylori proportion

In dataset SRP070925 high (>50%) *H. pylori* proportion is not detected, contrary to the healthy cohort SRP200169. In datasets SRP128749 and SRP172818, high *H. pylori* proportions are detected in subsets of all three micro-environments normal, peripheral and tumor. *H. pylori* free samples are found in all datasets, indistinctive of

disease progress or micro-environment.

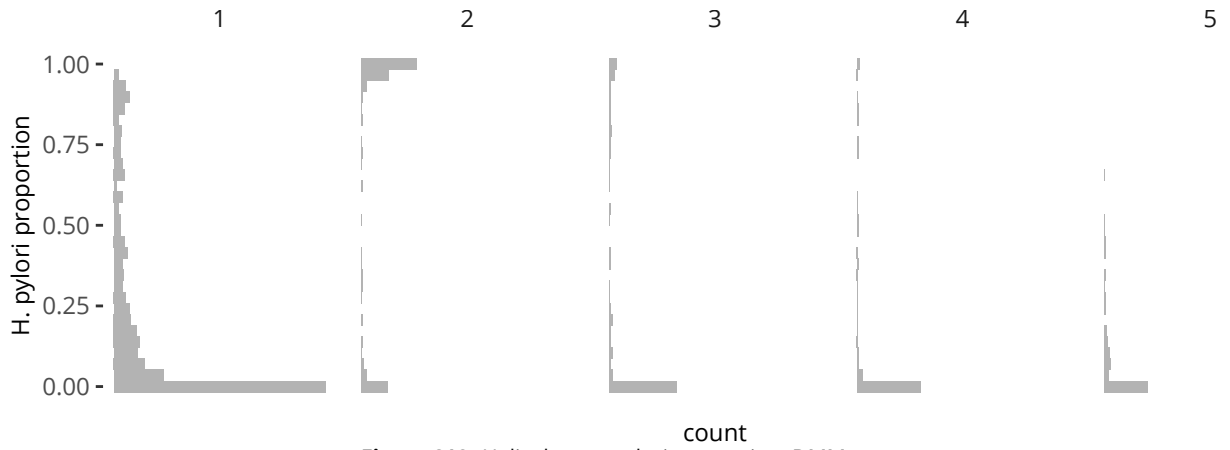


Figure S12: Helicobacter pylori proportion, DMMs.

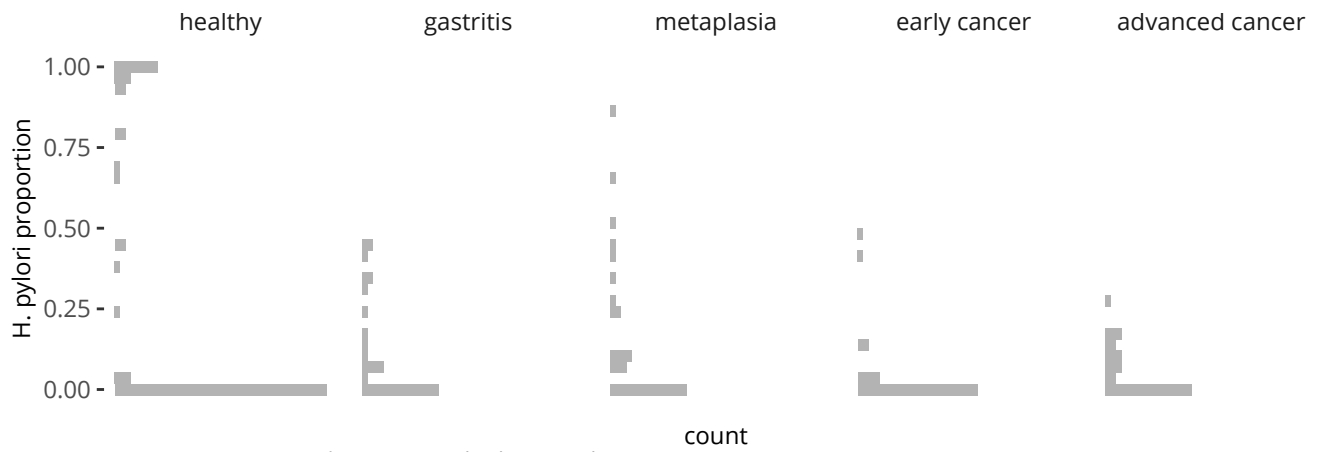


Figure S13: Helicobacter pylori proportion, SRP200169 + SRP070925.

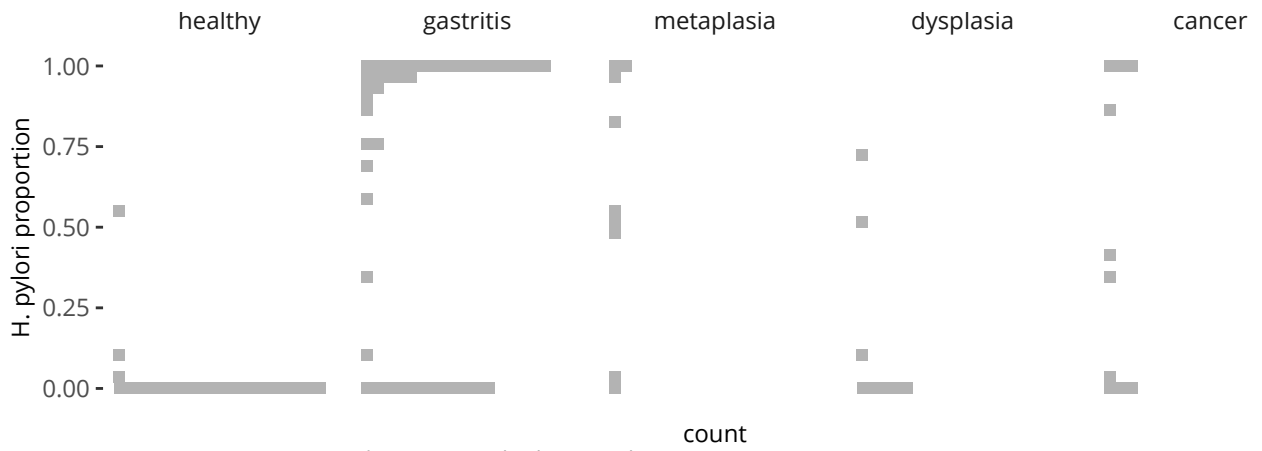
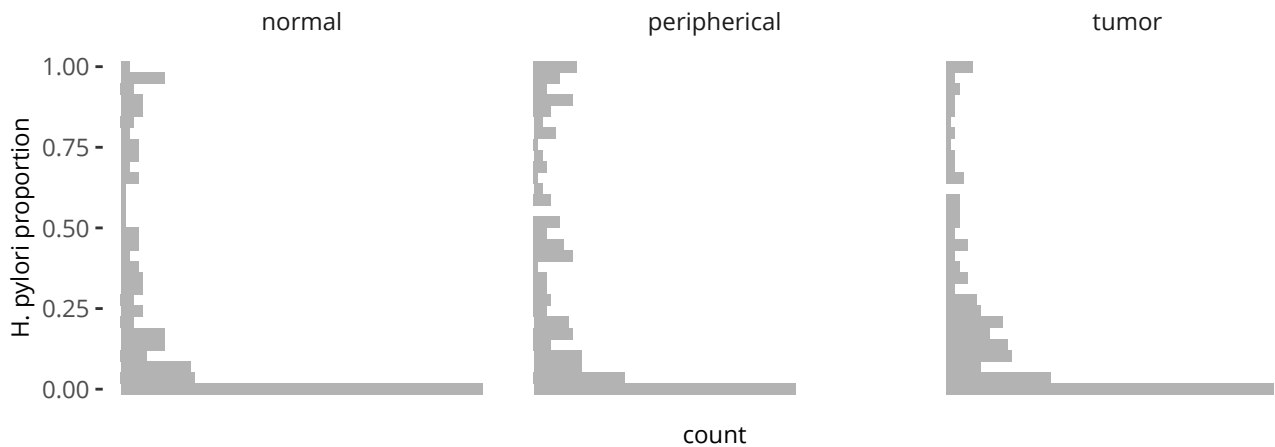
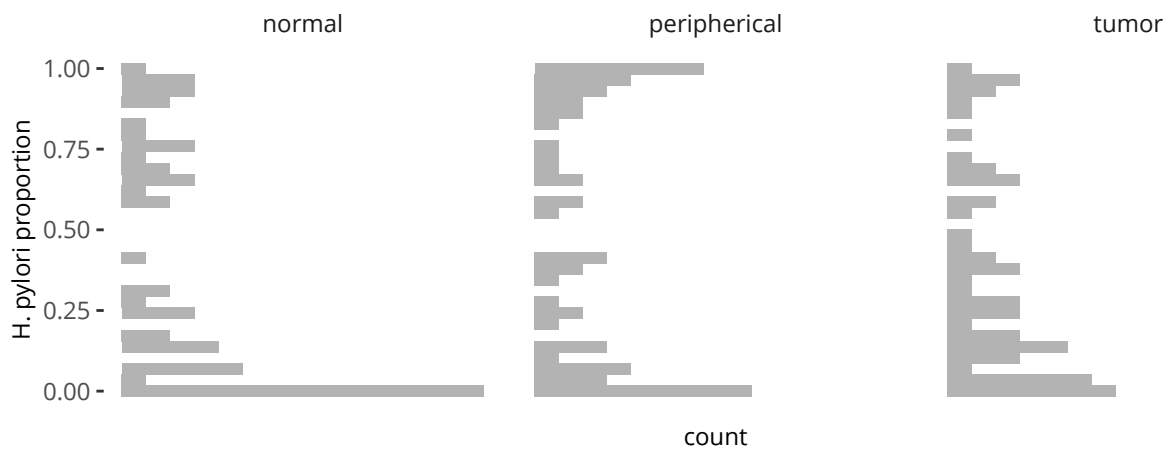


Figure S14: Helicobacter pylori proportion, ERP023334.



**Figure S15:** Helicobacter pylori proportion, SRP128749.



**Figure S16:** Helicobacter pylori proportion, SRP172818.

### Bibliography

Vanessa L Hale, Patricio Jeraldo, Jun Chen, Michael Mundy, Janet Yao, Sambhawa Priya, Gary Keeney, Kelly Lyke, Jason Ridlon, Bryan A White, Amy J French, Stephen N Thibodeau, Christian Diener, Osbaldo Resendis-Antonio, Jaime Gransee, Tumpa Dutta, Xuan-Mai Petterson, Jaeyun Sung, Ran Blekhman, Lisa Boardman, David Larson, Heidi Nelson, and Nicholas Chia. Distinct microbes, metabolites, and ecologies define the microbiome in deficient and proficient mismatch repair colorectal cancers. *Genome Medicine*, 10(1):78–13, October 2018a. doi: 10.1186/s13073-018-0586-6.

Vanessa L Hale, Patricio Jeraldo, Michael Mundy, Janet Yao, Gary Keeney, Nancy Scott, E Heidi Cheek, Jennifer Davidson, Megan Greene, Christine Martinez, John Lehman, Chandra Pettry, Erica Reed, Kelly Lyke, Bryan A White, Christian Diener, Osbaldo Resendis-Antonio, Jaime Gransee, Tumpa Dutta, Xuan-Mai Petterson, Lisa Boardman, David Larson, Heidi Nelson, and Nicholas Chia. Synthesis of multi-omic data and community metabolic models reveals insights into the role of hydrogen sulfide in colon cancer. *METHODS*, 149:59–68, October 2018b. doi: 10.1016/j.ymeth.2018.04.024.

Xiaosun Liu, Li Shao, Xia Liu, Feng Ji, Ying Mei, Yiwen Cheng, Fengping Liu, Chongxian Yan, Lanjuan Li, and Zongxin Ling. Alterations of gastric mucosal microbiota across different stomach microhabitats in a cohort of 276 patients with gastric cancer. *EBioMedicine*, 40:336–348, February 2019. doi: 10.1016/j.ebiom.2018.12.034.

Rachel V Purcell, Martina Visnovska, Patrick J Biggs, Sebastian Schmeier, and Frank A Frizelle. Distinct gut microbiome patterns associate with consensus molecular subtypes of colorectal cancer. *Scientific Reports*, 7(1):11590, September 2017. doi: 10.1038/s41598-017-11237-6.