Comparison of the human gastric microbiota in hypochlorhydric states arising as a result of *Helicobacter pylori*-induced atrophic gastritis, autoimmune atrophic gastritis and proton pump inhibitor use

Bryony N. Parsons[1#], Umer Zeeshan Ijaz[2#], Rosalinda D'Amore[3,] Michael D. Burkitt[1,7], Richard Eccles[3], Luca Lenzi[3], Carrie A. Duckworth[1], Andrew R. Moore[1,7], Laszlo Tiszlavicz[6], Andrea Varro[1], Neil Hall[3,4,5] and D. Mark Pritchard[1,7*]

[1]*Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Crown Street, Liverpool, L69 3GE, UNITED KINGDOM.*

[2]*University of Glasgow, Rankine Building, School of Engineering, Oakfield Avenue, Glasgow G12 8LT, UNITED KINGDOM*

[3]*Centre for Genomic Research, Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool, L69 7ZB, UNITED KINGDOM.*

[4]*The Earlham Institute Norwich Research Park Norwich, NR4 7UH*

[5]*School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, Norfolk, NR4 7TJ, UNITED KINGDOM*

[6]*Department of Pathology, University of Szeged, HUNGARY*

[7]*Royal Liverpool and Broadgreen University Hospitals NHS Trust, Prescot St, Liverpool, L7 8XP, UNITED KINGDOM*

* Corresponding author D. Mark Pritchard Tel: +44 151 794 5772 Email: dmpritch@liverpool.ac.uk

23 # These authors contributed equally

24 **Key words**: Gastric, hypochlorhydria, PPI, microbiota, atrophy, Helicobacter

25

26

27

ABSTRACT

Objective: Several conditions associated with reduced gastric acid secretion confer an altered risk of developing a gastric malignancy. *Helicobacter pylori*-induced atrophic gastritis predisposes to gastric adenocarcinoma, autoimmune atrophic gastritis is a precursor of type I gastric neuroendocrine tumours, whereas proton pump inhibitor (PPI) use does not affect stomach cancer risk. We hypothesised that each of these conditions was associated with specific alterations in the gastric microbiota and that this influenced subsequent tumour risk.

Design: 95 patients (in groups representing normal stomach, PPI treated, *H. pylori* gastritis, *H. pylori*-induced atrophic gastritis and autoimmune atrophic gastritis) were selected from a cohort of 1400. RNA extracted from gastric corpus biopsies was analysed using 16S rRNA sequencing (MiSeq).

Results: Samples from normal stomachs and patients treated with PPIs demonstrated similarly high microbial diversity. Patients with autoimmune atrophic gastritis also exhibited relatively high microbial diversity, but with samples dominated by *Streptococcus*. *H. pylori* colonisation was associated with decreased microbial diversity and reduced complexity of co-occurrence networks. *H. pylori*-induced atrophic gastritis resulted in lower bacterial abundances and diversity, whereas autoimmune atrophic gastritis resulted in greater bacterial abundance and equally high diversity compared to normal stomachs. Pathway analysis suggested that glucose-6-phospahte1-dehydrogenase and D-lactate dehydrogenase were over represented in *H. pylori-i*nduced atrophic gastritis versus autoimmune atrophic gastritis, and that both these groups showed increases in fumarate reductase.

Conclusion: Autoimmune and *H. pylori*-induced atrophic gastritis were associated with different gastric microbial profiles. PPI treated patients showed relatively few alterations in the gastric microbiota compared to healthy subjects.

SIGNIFICANCE OF THIS STUDY

1. What is already known about this subject?

- Some conditions which result in reduced gastric acid secretion and hypochlorhydria are associated with an increased risk of gastric tumourigenesis.
- This risk is different in patients with *H. pylori*-induced atrophic gastritis, autoimmune atrophic gastritis and chronic proton pump inhibitor use.
- Hypochlorhydria and *H. pylori* infection cause alterations in the composition of the gastric microbiota.

2. What are the new findings?

- We used 16S rRNA sequencing to characterise the microbiota in gastric corpus biopsies from a well characterised cohort of patients.
- The gastric microbiota was different in patients who were hypochlorhydric as a result of *H. pylori*-induced atrophic gastritis, autoimmune atrophic gastritis and proton pump inhibitor use.
- Biochemical pathways associated with gastric carcinogenesis such as the fumarate reductase pathway were predicted to be altered in patients with atrophic gastritis.

3. How might it impact on clinical practice in the foreseeable future?

- Understanding how the microbiota that colonise the hypochlorhydric stomach influence gastric carcinogenesis may ultimately permit stratification of patients' subsequent tumour risk.
- Interventions that alter the composition of the gastric microbiome in hypochlorhydric patients with atrophic gastritis should be tested to investigate whether they alter the subsequent risk of developing gastric malignancy.

INTRODUCTION

Gastric adenocarcinoma is the third most common cause of cancer related mortality worldwide[1] and most cases are associated with chronic *Helicobacter pylori* infection. Gastric cancer usually develops via the premalignant condition of gastric atrophy, which is associated with the loss of acid-secreting parietal cells[2]. The resulting hypochlorhydria potentially leads to alterations in the composition of the gastric microbiota by providing a more favourable environment for colonisation. It is currently unclear to what extent the non-*H. pylori* gastric microbiota contributes towards gastric carcinogenesis. Although the hypochlorhydria associated with autoimmune atrophic gastritis also increases the risk of developing gastric adenocarcinoma[3], it is more frequently associated with the development of another tumour, the type I gastric neuroendocrine tumour (NET)[4]. However, hypochlorhydria does not always increase the risk of gastric tumour development, as observed following chronic proton pump inhibitor (PPI) use[5]. Therefore, factors in addition to hypochlorhydria affect gastric cancer risk and one of these could be the gastric microbiota.

Although originally thought to be sterile, several bacterial communities have been shown to survive in the normal human stomach[6]. Differences have also been observed depending upon *H. pylori* status[6].There is now overwhelming evidence that certain bacteria influence cancer development. Potential mechanisms include altering the host immune system, exacerbating inflammation, or converting dietary nitrates to produce carcinogens such as N-nitrosamines and nitric oxide[7, 8, 9, 10, 11, 12, 13].

We therefore hypothesised that three stimuli which result in hypochlorhydria, namely *H. pylori*-induced atrophic gastritis, autoimmune atrophic gastritis and proton pump

inhibitor use cause specific changes to the composition of the gastric microbiota. In addition, the gastric microbiota that is present in these conditions contributes towards the specific gastric tumour risk that is associated with each of these hypochlorhydric states. We have used 16S rRNA sequencing to determine the gastric mucosal microbiota profiles in patients with these causes of hypochlorhydria and have compared these with samples obtained from healthy subjects and from patients with *H. pylori*-induced gastritis, but no evidence of gastric atrophy.

METHODS

Ethics

Acquisition of the biopsies used in this study was approved by Liverpool (08/H1005/37) and Cambridge East (10/H0304/51) Research Ethics Committees as previously described[14, 15]. All patients gave written informed consent.

Patients

One hundred gastric biopsy samples in 5 different groups were selected from a cohort of 1400 prospectively recruited patients who underwent diagnostic upper gastrointestinal endoscopy at Royal Liverpool University Hospital[14] and from 8 patients with type I gastric NETs who had been recruited to a clinical trial[15, 16] (Table 1).

Table 1. Summary of patient group characteristics

	Group	Total no. of samples	Number of females	Age (years)		BMI		*H. pylori* serology +ve	*H. pylori* histology /urease +ve	PPI use	Atrophic gastritis	Serum gastrin (pM)		Anti GPC/IF antibody
				Median	IQR	Median	IQR					Median	IQR	
1	Normal	20	13	46	30.5-58.7	24.5	21.2-26.8	0	0	-	-	22.5	17.5-28.5	- or ND
2	PPI treated	19	13	60	46-67	28.5	26.1-33.2	0	0	+	-	140	94-200	- or ND
3	*H. pylori* gastritis	22	11	57.5	47.7-64	27.4	23.3-27.1	22	22	-	-	21.5	17.4-28	- or ND
4	*H. pylori* atrophy	23	15	65	55-72	25.9	24.6-31.9	23	6	-	+	100	64-260	- or ND
5	Autoimmune atrophy	11	6	67	60-76	28.6	23.7-35	2	0	-	+	800	470-1050	+

ND= Not done

IQR= Interquartile range, 25% and 75%

Patients in the normal stomach group had a normal endoscopy, no evidence of *H. pylori* infection by histology, rapid urease test or serology, were not taking a PPI and were normogastrinaemic. Patients belonging to the *H. pylori* gastritis group were positive for *H. pylori* by rapid urease test, histology and serology, had no histological evidence of atrophic gastritis, were not taking a PPI and were normogastrinaemic. Patients in the *H. pylori*-induced atrophic gastritis group showed histological evidence of corpus atrophic gastritis and/or intestinal metaplasia, had no dysplasia or cancer, were positive for *H. pylori* by serology, were not taking a PPI and were hypergastrinaemic. Six out of the 23 patients in this group were also *H. pylori* positive by urease test and/or histology. Patients in the autoimmune atrophic gastritis group had histological evidence of atrophic gastritis, no evidence of *H. pylori* infection by rapid urease test or histology, positive anti-gastric parietal cell and/or intrinsic factor antibodies, were markedly hypergastrinaemic and 8 out of 11 also had grade 1 type I gastric NETs. Patients in the PPI-treated group were currently taking PPIs, had no evidence of *H. pylori* infection by serology, rapid urease test or histology, had no histological evidence of atrophic gastritis and were hypergastrinaemic (suggesting significant hypochlorhydria).

Samples

At least two biopsies per site were obtained from the gastric antrum and corpus for histopathology. Eight additional corpus biopsies were stored in RNA later immediately after removal and were extracted using a modified Tri- reagent protocol[17]. Briefly, samples were thawed and separated from RNA later, before being homogenised in Tri-Reagent® (Sigma-Aldrich, Gillingham, UK). Chloroform was added and the resulting clear aqueous layer was combined with isopropanol before centrifugation to produce a precipitated RNA pellet. This was washed with

75% and 100% ice cold ethanol before being allowed to dry and then resuspended in diethylpyrocarbonate (DEPC)-treated water (Sigma-Aldrich, Gillingham, UK). RNA was stored in ethanol at -80°C. Ethanol was removed and pellets were resuspended in DEPC-water prior to reverse transcription.

Gastrin assays

Serum gastrin concentrations were measured by radioimmunoassay (RIA) as previously described[18, 19]. Fasting serum gastrin concentrations were all <40pM in normogastrinaemic subjects and >40pM (with the majority >100pM) in hypergastrinaemic subjects.

Reverse Transcription

Samples and random primers were denatured together for 5 minutes at 65°C before Proto reaction mix and Proto enzyme from a ProtoScript® II First Strand cDNA Synthesis kit (NEB, E6560L) were added. Samples were then incubated at 25°C for 5 minutes, 42°C for 20 minutes, and 80°C for 5 minutes. Newly synthesised cDNA was then measured using a Qubit high sensitivity assay (ThermoFisher Ltd, Paisley, UK).

16S rRNA Sequencing

The 16S rRNA gene was targeted using V1-V2 (27F and 388R) primers[20] with slight modifications: forward primer 5'ACACTCTTTCCCTACACGACGC TCTTCCGATCTNNNNNAGAGTTTGATCMTGGCTCAG'3, reverse primer 5'GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGCCTCCCGTAGGAGT' 3. Primers were validated using a mock community described in supplementary methods. The following cycling conditions were used: initial denaturation 94°C for 5

minutes, followed by 10 cycles of denaturation at 98°C for 20 seconds, annealing at 60°C for 15 seconds, and elongation at 72°C for 15 seconds, followed by a final elongation step of 72°C for 1 minute. PCR amplicons were purified to remove excess primers, nucleotides, salts, and enzymes using the Agencourt® AMPure® XP system (Beckman Coulter Ltd, High Wycombe, UK). Purified amplicons were used in a second PCR reaction with the same conditions except with 20 cycles. This second step was used to add dual index barcodes. The PCR amplicons were purified as above. All PCR reactions used Kapa HiFi HotStartStart 2× master mix (Anachem Ltd, Bedfordshire, UK) and all primers were used at 10µM. Amplicon sizes were checked using a fragment analyser (Advanced Analytical, Ankeny, USA) and size selection was performed using a Pippin prep (Sage Science, Beverly, USA). The quantity and quality of the samples in the final libraries were checked using a SYBR Green qPCR assay and the Illumina Library Quantification kit (Kapa) on a Roche Light Cycler LC480II, according to the manufacturer's instructions.

Prior to loading samples onto the MiSeq, PhiX was added (10-15%) to increase diversity, and samples were then denatured with NaOH according to the Illumina MiSeq protocol. ssDNA library fragments were diluted to a final concentration of 8pM. 600µl of ssDNA library was loaded into a MiSeq Reagent Cartridge and a 500–cycle PE kit v2 was used. Paired-end sequencing was performed according to the manufacturer’s instructions (Illumina, SanDiego, CA, USA). Sequence analysis methodology is described in the supplementary methods. Reads were submitted to EBI short-read archive accession-PRJEB21104.

Statistical analysis

Details are described in the supplementary methods.

RESULTS

Patient characteristics

Patients were selected from the larger cohort according to criteria defined above. Characteristics of the selected patients are shown in table 1 and figure 1A. One sample from the normal stomach group and four from the PPI-treated group were subsequently excluded because sequencing showed the presence of >15% *H. pylori* despite this organism being undetected by conventional clinical tests (most likely due to the higher sensitivity of 16S rRNA sequencing compared to routine clinical tests). Ninety-five samples were therefore analysed. Negative extracts from the RNA extraction procedures, a water sample in the first PCR and a mock bacterial community were also sequenced.

Detection of Operational Taxonomic Units (OTUs)

In total 10,386 OTUs were identified. Extraction controls contained fewer OTUs than the patient samples, whilst the mock communities (and a random selection of 10 gastric samples – data not shown) showed consistency between MiSeq runs (Figs 1-3). Despite the negative extracts being theoretically sterile, as expected they generated 16S signals due to known background reagent contamination[21]. Samples from the autoimmune atrophic gastritis group contained the largest number of OTUs, whilst all other patient groups were comparable (Fig 1B). Mock communities demonstrated the expected bacterial ratios (Fig 3B).

Bacterial diversity and abundance in the different hypochlorhydric states

Twenty-three known phyla were identified, mainly Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria and Cyanobacteria. Bacteroidetes, followed by Proteobacteria and Firmicutes were most common in normal stomachs, whereas samples from PPI-treated patients contained slightly more Firmicutes and fewer Bacteroidetes. The *H. pylori* gastritis and *H. pylori* atrophic gastritis samples were dominated by Proteobacteria (as *Helicobacter* itself is a member of this phylum), whilst biopsies from patients with autoimmune atrophic gastritis contained the largest proportion of Firmicutes compared to all other patient groups.

Alpha diversity

Diversity indices demonstrated that the microbiota in normal stomachs was significantly more diverse than in the stomachs of all other patient groups except for the patients who had autoimmune atrophic gastritis (Fig 2). Evaluation of evenness (by Pielou's evenness and Simpson) suggested that the samples from normal stomachs and from the stomachs of patients taking PPIs contained bacterial communities that were more equal in abundance than those in the other patient groups, which were more skewed (Fig 2). Calculations based on richness indicated that the samples from normal stomachs also contained the greatest number of different bacterial species compared to all other groups, whilst the two *H. pylori* infected groups (*H. pylori*-induced gastritis and atrophy) contained significantly fewer species.

246 *Beta diversity*

247 When beta diversity was explored using nonmetric distance scaling (NMDS), patient
248 groups clustered predominantly by bacterial abundance (Fig 4). When *H. pylori* was
249 removed from the analysis however, *H. pylori* gastritis patients no longer clustered
250 separately by abundance from subjects with normal stomachs (Fig 4). Despite this,
251 following removal of *H. pylori*, there was a significant difference in abundance for *H.*
252 *pylori*-induced atrophic gastritis patients compared to normal stomachs, suggesting
253 strongly that there are differences in the proportions of non-*H. pylori* bacteria in these
254 subjects compared with others (Fig 4). Samples from patients who had autoimmune
255 atrophic gastritis displayed the only significant differences in terms of the presence
256 or absence of specific bacteria compared to other groups (Fig 4). This suggests that
257 changes in the gastric bacterial community during hypochlorhydria usually involve
258 changes in the relative proportions of bacteria that are already present, and only
259 rarely involve the loss or gain of specific bacterial genera.

260

261 **Comparisons between the microbiota profiles in the different patient groups**
262 **and healthy controls**

263 *Normal stomach versus PPI treated patients*

264 Patients receiving PPIs showed similar bacterial profiles to those found in the
265 stomachs of normal subjects, despite having significantly higher serum gastrin
266 concentrations (suggesting the presence of hypochlorhydria) (Figs 1A, 3 & S1).
267 Nonetheless there were differences in the ranks of most abundant bacterial families.
268 In normal (control) patients Prevotellaceae were the most abundant bacterial family

(23%), followed by Streptococcaceae (10%), Paraprevotellaceae (7%) and Fusobacteriaceae (5%); amongst PPI-treated individuals Streptococcaceae (17%) outranked Prevotellaceae (11%), Campylobacteraceae (5%) and Leptotrichiaceae (4%; Fig 3A). The only significant differences at genus level between these groups were decreases in *Actinobacillus* and *Tannerella* in the PPI-treated stomachs (Table S1).

Very few differences were identified in co-occurrence network analyses when the microbiota in normal stomachs was compared to PPI-treated stomachs. The only observed difference was a negative correlation between *Helicobacter* and *Acinetobacter* in the PPI-treated samples, whereas this relationship was positively correlated in normal stomach biopsies (Figs 5A, S2A & Table S2A). Predicted pathway analysis showed no significantly different biochemical pathways between these two groups (Table S3).

Normal stomach versus H. pylori-induced gastritis

Unsurprisingly, the microbiota in the stomachs of patients who had *H. pylori*-induced gastritis consisted almost entirely of Helicobacteraceae (97%) (Fig 3). When compared to normal patients, *H. pylori*-induced gastritis patients showed a greater number of differences at the genus level than all other patient groups (Table S1). The majority of these differences resulted from reductions in the proportions of several bacterial genera within the *H. pylori* gastritis group. To ensure that the dominance of *H. pylori* did not skew the proportions of the other bacteria in a misrepresentational way, *H. pylori* OTUs were removed from the abundance table followed by differential expression analysis on the remaining raw abundances. This

293 analysis resulted in almost identical results to when *H. pylori* remained (Table S1).
294 Due to the dominance of *H. pylori* in these patients, very few co-occurrence networks
295 were identified, but positive correlations were observed between *Kocuria* and
296 *Skermanella* in both groups (Figs 5A & B). Predicted pathway analysis suggested a
297 reduction in several dehydrogenases in the stomachs of patients who had *H. pylori*
298 gastritis (Table S3).

299

300 *Normal stomach versus H. pylori-induced atrophic gastritis*

301 The stomachs of patients who had *H. pylori*-induced atrophic gastritis were also
302 dominated by Helicobacteraceae (62%), followed by Streptococcaceae (5%),
303 Fusobacteriaceae (2%) and Prevotellaceae (2%) (Fig 3). At the genus level, several
304 differences were observed between normal stomachs and the stomachs of patients
305 with *H. pylori*-induced atrophic gastritis. These included decreases in the proportions
306 of *Tannerella (E. coli/Shigella/Salmonella), Treponema,* and *Prevotella* in the *H.*
307 *pylori*-induced atrophic gastritis group. The vast majority of these differences
308 remained when *H. pylori* was removed from the analysis (Table S1). Prevotellaceae
309 were generally lower in all patient groups compared to normal stomachs (Figs S1
310 and 3). As with the *H. pylori* gastritis group, the majority of these changes reflected
311 decreases in the proportions of various bacterial genera within the *H. pylori*-induced
312 atrophic gastritis group, with the only increase being in *Helicobacter* itself.

313 Co-occurrence networks were more complicated in *H. pylori*-induced atrophic
314 gastritis patients compared to those subjects who had *H. pylori*-induced gastritis
315 (Figs 5B &D). Clear negative relationships were observed between *Helicobacter* and
316 genera such as *Streptococcus,* whilst *Campylobacter, Prevotella, Haemophilus* and

317 *Veillonella* were amongst the most well-connected and influential bacteria observed
318 in the stomachs of *H. pylori* atrophic gastritis patients (Fig 5 and Table S2B).
319 Predicted pathway analysis showed that several pathways were under-represented
320 in the *H. pylori*-induced atrophic gastritis group, including succinate dehydrogenase
321 (Table S3). Over-represented pathways included fumarate reductase (Table S3).

322

323 *Normal stomach versus autoimmune atrophic gastritis*

324 Streptococcaceae (38%) were the most dominant group identified in the stomachs of
325 patients who had autoimmune atrophic gastritis, followed by Prevotellaceae (9%),
326 Flavobacteriaceae (7%), Campylobacteraceae (7%), Enterobacteriaceae (5%) and
327 Pasteurellaceae (5%). The stomachs of autoimmune atrophic gastritis patients
328 contained a higher proportion of Streptococcaceae than all other patient groups (Fig
329 3) and were the only samples that showed complete loss or gain of bacteria rather
330 than simply changes in bacterial proportions (Fig 4D). For example, the stomachs of
331 autoimmune atrophic gastritis patients were colonised by *Gemella* and *Bosea* unlike
332 any other patient group. Alterations in the relative proportions of other bacteria were
333 also found in the stomachs of patients with autoimmune atrophic gastritis. These
334 included increases in the proportions of *Streptococcus*, *Campylobacter and*
335 *Haemophilus* (Table S1).

336 Few co-occurrence networks were identified, presumably due to the dominance of
337 Streptococcaceae, although *Stenotrophomonas* and *Delftia*; and *Selenomonas* and
338 *Pseudomonas* showed strong positive correlations (Fig 5D and Table S2B).
339 Predicted pathway analysis suggested that several pathways were over- or under-

represented in the stomachs of patients who had autoimmune atrophic gastritis (table S3).

H. pylori gastritis versus H. pylori-induced atrophic gastritis

We investigated whether the microbiota in the stomachs of patients who had *H. pylori*-induced atrophic gastritis (which were likely to be hypochlorhydric as indicated by hypergastrinaemia) differed from that in patients who had *H. pylori* gastritis, normal gastric acid secretion and normogastrinaemia. No significant differences were identified at the genus level. However, several OTUs belonging to *H. pylori* were found more frequently in the *H. pylori*-induced atrophic gastritis group, possibly suggesting the presence of particular strains within this group (Table S4A). Interestingly, only two other OTUs differed in abundance between these groups, *Streptococcus mitis* and *Neisseria mucosa.* However, these did not remain significant once *H. pylori* was removed from the analysis. This suggests that the presence of atrophy does not result in extensive changes to bacterial communities in the stomach relative to the simple presence of *H. pylori*, but may result in specific differences in individual bacterial strains.

Comparisons between the gastric microbiota of individuals with hypochlorhydria of different aetiologies

Patients with *H. pylori*-induced atrophic gastritis and those receiving PPIs had similar fasting serum gastrin concentrations (median 100pM and 140pM respectively), possibly suggesting similar degrees of hypochlorhydria (although *H. pylori* infection

363 may have directly contributed to the hypergastrinaemia in the former group). In
364 contrast patients with autoimmune atrophic gastritis were associated with higher
365 fasting serum gastrin concentrations (median 800pM; Table 1). No direct association
366 between fasting serum gastrin concentration and bacterial taxa was observed
367 between the different groups (PERMANOVA Unifrac P=0.512, weighted Unifrac
368 P=0.721 and Bray-Curtis P=0.556). This is reflected in the evidence that patients
369 with *H. pylori*-induced atrophic gastritis and those receiving PPIs exhibited marked
370 differences in 16S rRNA microbiota profiles, co-occurrence networks and predicted
371 pathways, despite similar gastrin levels. And that patients with autoimmune atrophic
372 gastritis showed similarities to individuals with *H. pylori*-induced atrophic gastritis by
373 predicted pathway analysis, despite markedly different serum gastrin concentrations
374 (Table S3).

375 Samples from patients with autoimmune atrophic gastritis contained significantly
376 more *Streptococci* than all other groups (Fig 3 & Table S1). *Streptococcus* did not
377 appear to be similarly increased in *H. pylori*-induced atrophic gastritis; this may have
378 been due to the negative relationship observed between *Helicobacter* and
379 *Streptococcus* identified in co-occurrence networks (Fig 5C).

380 Gastric biopsies from patients with autoimmune atrophic gastritis and those on PPIs
381 both showed greater bacterial diversity than was observed in the stomachs of
382 patients with *H. pylori*-induced atrophic gastritis (Fig 2). At the genus level, patients
383 with autoimmune atrophic gastritis showed significant increases in *Tannerella*,
384 *Dorea*, *Streptococcus, Fusobacterium* and *Campylobacter* compared to the patients
385 with *H. pylori*-induced atrophic gastritis (Table S4B). The stomachs of PPI-treated
386 patients also contained significantly higher proportions of *Fusobacterium* and
387 *Campylobacter* than the stomachs of *H. pylori*-induced atrophic gastritis patients.

Furthermore, patients receiving PPI treatment showed significantly higher proportions of *Flavisolibacter* and *Dermacoccus* in their stomachs than autoimmune atrophic gastritis patients, but significantly less *Paludibacter, Granulicatella*, *Streptococcus*, and *Neisseria*.

Patients who had atrophic gastritis due to *H. pylori* or an autoimmune aetiology both showed over-representation of several mutual pathways compared to controls (Table S3). However, differences between the two groups were also observed. For example, glucose-6-phosphate 1−dehydrogenase and D−lactate dehydrogenase pathways were over-represented in the stomachs of patients who had *H. pylori*-induced atrophic gastritis compared to those who had autoimmune atrophic gastritis (Table S3).

DISCUSSION

Gastric samples obtained from subjects who had a normal stomach, no evidence of *H. pylori* infection and normogastrinaemia had the highest levels of microbial diversity. This is consistent with other reports of healthy populations showing more microbial diversity[22, 23, 24]. These samples also contained the greatest proportion of Prevotellaceae (23%) which corroborates previous research that reported normal stomachs contained 37% *Prevotella*, reducing to 28% in dyspeptic patients[25]. In general, the microbiota, co-occurrence networks and predicted pathways in samples from PPI-treated patients were similar to those in normal stomachs. This agrees with other reports that PPIs do not significantly influence the gastric microbiota[26, 27]. At the OTU level however, samples from PPI-treated patients contained significantly more *Streptococcus.* This has also been observed in gastric[27] and faecal samples from twins discordant for PPI use[28].

H. pylori gastritis, and to some extent *H. pylori*-induced atrophic gastritis samples were dominated by *H. pylori.* This observation may have been exacerbated by our use of RNA as opposed to DNA for sequencing, unlike many other publications. When these two techniques were directly compared, *H. pylori* abundance was found to be 19.9 times higher in RNA compared to DNA from gastric fluid samples, and was also more dominant in biopsies than gastric fluid[26, 29]. The use of RNA ensured that only viable bacteria were included in the analysis, giving a better indication of the taxa that are likely to be influencing the gastric environment. *H. pylori* colonisation was associated with a decrease in gastric bacterial diversity, and dominance of this organism, which is highly adapted to the gastric environment, has also been reported previously[6, 30, 31].

The majority of changes observed in *H. pylori* gastritis and *H. pylori*-induced atrophic gastritis samples were due to reductions in non-*H. pylori* bacteria. *H. pylori*-induced atrophic gastritis samples showed complex co-occurrence networks, unlike *H. pylori* gastritis which showed few connections, presumably related to the dominance of *H. pylori* itself in those samples. *Campylobacter, Prevotella, Haemophilus* and *Veillonella* were amongst the most influential genera in *H. pylori*-induced atrophic gastritis samples. These bacteria have been previously identified in oral and gastric samples[29].The only differences found between the two *H. pylori* patient groups at the OTU level were increased abundances of specific *H. pylori* OTUs (possibly suggesting specific bacterial strains) and increased proportions of *Streptococcus mitis* and *Neisseria mucosa* in the atrophic group. The former species and latter genus have been identified from oral microbiota as potential biomarkers for pancreatic cancer[32]. *Neisseria* has been shown to produce large amounts of alcohol dehydrogenase, which produces the carcinogen acetaldehyde, and along with *H. pylori*'s high production of this enzyme, may also contribute to gastric carcinogenesis[33]. Some strains of Streptococcaceae have previously been shown to affect the outcomes of *H. pylori* infection. For example, *S. mitis* induces a coccoid state in *H. pylori*[34] and this may lead to unsuccessful antibiotic treatment and false negative diagnostic test results. Moreover, this coccoid form has been suggested to be more associated with gastric adenocarcinoma development than the spiral form[35, 36].

The stomachs of patients with autoimmune atrophic gastritis (who probably had the most profound reductions in acid secretion, as suggested by higher fasting serum gastrin concentrations), showed high bacterial diversity. Samples from this group also showed significantly higher proportions of *Streptococcus* than any of the other

451 groups. They also contained *Ruminococcus* and *Gemella* unlike any other patient
452 group except *H. pylori*-induced atrophic gastritis, although they did not contain
453 genera such as *Arthrobacter, Cupriavidus* and *Sneathia*. Therefore, bacterial
454 communities were both lost and gained in this condition. Co-occurrence networks
455 appeared to be disrupted by the overabundance of Streptococcaceae resulting in
456 few connections.

457 The microbial profiles in the stomachs of patients with *H. pylori*-induced atrophic
458 gastritis and autoimmune atrophic gastritis were quite different. In addition, pathways
459 such as glucose-6-phosphate 1−dehydrogenase and D−lactate dehydrogenase were
460 over-represented in the stomachs of patients with *H. pylori*-induced atrophic gastritis
461 compared to autoimmune atrophic gastritis. Overexpression of these pathways has
462 been associated with poorer prognoses in gastric cancer[37, 38]. Conversely,
463 several other metabolic pathways such as fumarate reductase were increased in
464 representation in patients with both autoimmune and *H. pylori* associated atrophic
465 gastritis. Fumarate reductase is involved in the metabolism of some bacteria and is
466 essential for colonisation by *H. pylori* in the mouse stomach[39, 40, 41]. Interestingly,
467 succinate dehydrogenase (which has an opposite action to fumarate reductase) was
468 found to be decreased in both atrophic gastritis groups compared to both the normal
469 and PPI-treated samples. Lower levels of succinate dehydrogenase have previously
470 been found in gastrointestinal tumours and parietal cells[42, 43]. PPI-treated patients
471 showed more similarities in microbial diversity and abundance to the patients who
472 had autoimmune atrophic gastritis, than to the patients who had *H. pylori*-induced
473 atrophic gastritis.

474

Conclusion

Our findings indicate that *H. pylori* colonisation and hypochlorhydria result in changes in gastric bacterial abundance and only rarely in loss/gain of bacteria. PPI treatment did not significantly alter the gastric microbiota from that of a normal stomach, despite serum gastrin concentrations being comparable to those found in patients with *H. pylori*-induced atrophic gastritis. Autoimmune atrophic gastritis resulted in a different, more diverse microbial pattern than that observed in the stomachs of patients who had *H. pylori*-induced atrophic gastritis. This may be due to differences in acid secretion between these conditions or other factors such as different immune profiles. Several biochemical pathways were represented in similar fashions in both atrophic gastritis groups. In particular, gastric-atrophy was associated with changes in the citric acid cycle (biochemical pathway that is known to be associated with gastric carcinogenesis) and our findings suggest that the microbiota may be an important contributor to this.

ACKNOWLEDGEMENTS

This study was funded by a grant from Worldwide Cancer Research 12-1028 to DMP, AV and NH. U.Z. Ijaz is funded by a NERC fellowship NE/L011956/1. MDB was funded by a CORE / British Society of Gastroenterology Development Grant and Wellcome Trust / University of Liverpool Institutional Strategic Support Fund grant under grant agreement number: 097826/Z/11/Z. Patients were recruited to the initial studies via grants from National Institute for Health Research (NIHR) via the Liverpool Biomedical Research Centre (BRC) and Trio Medicines Ltd.

CONFLICT OF INTERESTS

DMP has previously received research funding from Trio Medicines Ltd to investigate the treatment of gastric neuroendocrine tumours using Netazepide. None of the other authors has any conflict of interests.

AUTHOR CONTRIBUTIONS

BNP contributed to, analysis, interpretation and acquisition of data, drafting and critically appraising the manuscript. UZI performed and designed analysis, performed statistical analysis, interpreted data and critically appraised the manuscript. RD'A designed methodology, acquired data and critically appraised the manuscript. MDB contributed to analysis and interpretation of data, statistical analysis and critically appraising the manuscript. RE, CAD and ARM contributed to methodology, acquisition of data and critically appraising the manuscript. LT provided pathology expertise, interpretation of data and critically appraised the manuscript. AV, NH and DMP formulated conception of the study, its design, secured funding, interpreted

data and critically appraised the manuscript. All authors approved the final version of the manuscript and agree to accountability for all aspects of the work.

FIGURE LEGENDS

Table 1. Summary of patient group characteristics. ND = Not done, IQR = Interquartile range, 25% and 75%.

Figure 1. (A) Median fasting serum gastrin concentrations (pM) in patient groups. Kruskal-Wallis test with Dunn's comparison, plotted using Tukey's method *=P<0.05, and ****=P<0.0001 vs control. (B) Mean number of OTUs identified within each patient group, 1-way ANOVA and Tukey's multiple comparison test *=P<0.05, **=P<0.01, Control vs Autoimmune atrophic gastritis P=0.059, Control vs Neg P=0.061 and Hp-induced atrophic gastritis vs Neg P=0.059.

Figure 2. Five different diversity indices of human gastric microbiota (Fisher alpha: parametric index of diversity that models species as logseries distribution; Pielou's evenness: how close in numbers each species is; Richness: number of species per sample; Shannon: a commonly used index to characterise species diversity; and Simpson: which takes into account the number of species present, as well as their relative abundance). Pair-wise ANOVA was performed between different groups and if significant (P<0.001), the p-values have been drawn on top. Atrophy=*H. pylori* associated atrophy, Auto=autoimmune atrophic gastritis, Control=normal stomach, HP Gast=*H. pylori* associated gastritis, PPI=proton pump inhibitor and Neg= extraction control.

Figure 3. Relative abundances of taxa found within (A) groups and (B) individual human gastric biopsies. Hp=*H. pylori,* IM=intestinal metaplasia, IM+At=intestinal metaplasia and atrophy, PPI=proton pump inhibitor, EC=extraction controls (one of the EC samples was included in a run with more *H. pylori* dominant samples), H=H_2O and M=mock community (which showed consistent findings on two runs as

542 shown). All *H. pylori* atrophic gastritis samples were positive for *H. pylori* by serology,
543 + indicates whether these samples were also positive by histology/rapid urease test.
544 Autoimmune atrophic gastritis samples recorded as 's' were also positive for *H. pylori*
545 by serology.

546 **Figure 4**. Nonmetric distance scaling (NMDS) demonstrating clustering of patient
547 groups using (A) unweighted Unifrac distance (pair-wise distance between samples
548 is calculated as a normalised difference in cumulative branch lengths of the
549 observed OTUs for each sample on the phylogenetic tree without taking into account
550 their abundances in samples), (B) Bray-Curtis distance (abundance of OTUs alone
551 and not considering the phylogenetic distance) and (C) weighted Unifrac
552 (unweighted unifrac distance weighted by abundances of OTUs). Serum gastrin
553 concentration indicated by size of each point. Ellipses represent 95% CI of standard
554 error for a given group. Dotted ellipses represent the 95% CI of standard error when
555 *H. pylori* were removed from the analysis. Atrophy=*H. pylori* associated atrophic
556 gastritis, Auto=autoimmune atrophic gastritis, Control=normal, HP Gastr=*H. pylori*
557 associated gastritis and PPI=proton pump inhibitor. PERMANOVA (distances against
558 groups) suggests significant differences ($P<0.001$ for all three distances) in microbial
559 community explaining the following variations (R^2) between groups: 10% (8.6%
560 without *H. pylori* when using Unweighted Unifrac; 58% (14.5% without *H. pylori*)
561 when using Weighted Unifrac; and 15% when using Bray-Curtis distance. No
562 significant explanation was observed ($P>0.05$) for age, BMI, or serum gastrin
563 concentration in the PERMANOVA test. (D) Data from betadisper plots (a mean to
564 compare the spread/variability of samples for different groups) representing
565 difference in distances (Bray-Curtis, Unweighted and weighted Unifrac) of group
566 members from the centre/mean of individual groups after obtaining a reduced-order

567 representation of abundance table using Principle Coordinate Analysis. The pair-
568 wise differences in distances from group centre/mean were then subjected to
569 ANOVA and if significant (P<0.001), the p-values were drawn on top.

570 **Figure 5**. Co-occurrence network analysis between different genera (OTUs collated
571 together at genus level) when considering samples for (A) normal stomach, (B) *H.*
572 *pylori* gastritis, (C) *H. pylori*-induced atrophic gastritis and (D) autoimmune atrophic
573 gastritis. The genera were connected (Blue: positive correlation; Red: negative
574 correlation) when the pair-wise correlation values were significant (P.adj<0.05) after
575 adjusting the P values for multiple comparisons. Furthermore, subcommunity
576 detection was performed by placing the genera in the same subcommunity
577 (represented by colour of nodes) when many links were found at correlation values
578 >0.75 between members of the subcommunity. The size of the nodes represent the
579 degree of connections.

580

581 **Supplementary Figure and Table Legends**

582 **Figure S1**. Relative abundances of taxa found within human gastric biopsies after
583 removal of Helicobacteraceae. Hp=*H. pylori*, IM=intestinal metaplasia,
584 IM+At=intestinal metaplasia and atrophy, PPI=proton pump inhibitor. All *H. pylori*
585 atrophy samples were positive for *H. pylori* by serology.

586 **Figure S2**. Co-occurrence network analysis between different genera (OTUs
587 collated together at genus level) when considering samples for (A) PPI. The genera
588 were connected (Blue: positive correlation; Red: negative correlation) when the pair-
589 wise correlation values were significant (P.adj<0.05) after adjusting the P values for

multiple comparisons. Furthermore, subcommunity detection was performed by placing the genera in the same subcommunity (represented by colour of nodes) when many links were found at correlation values >0.75 between members of the subcommunity. The size of the nodes represent the degree of connections (B) network-wide statistics by degree, closeness, betweenness and eigenvalue centrality for *H. pylori* atrophic gastritis cases. The nodes (coloured with respect to subcommunity they are part of) were placed on concentric circles with values increasing from center to the periphery. A high betweenness for a node suggests many connections, whereas a high eigenvalue centrality suggests that those connections, in turn, are all well connected. On average a high betweenness and at the same time low eigenvalue centrality for a subcommunity suggests a keystone/important subcommunity.

Table S1. Significantly different genera identified between normal stomach samples and PPI, autoimmune atrophic gastritis, *H. pylori*-induced atrophic gastritis and *H. pylori* gastritis. The most significant species are identified at the top. Differential expression analysis based on the Negative Binomial (Gamma-Poisson) distribution and were corrected for multiple comparisons. * indicates a genus no longer significant when *H. pylori* was removed from the analysis.

Table S2A. Stable bacterial populations and correlations in PPI patients compared to other groups (if the correlation between two genera were consistently positive or negative in different groups). PPI versus *H. pylori*-induced atrophic gastritis in table S2B. No significant comparisons were found between PPI and autoimmune atrophic gastritis groups.

614 **Table S2B**. Stable bacterial populations and correlations in *H. pylori*-induced
615 atrophic gastritis patients compared to other groups.

616 **Table S3**. The top most significant predicted pathways found for each group
617 comparison.

618 **Table S4A**. Significant bacterial species identified between *H. pylori* atrophic gastritis
619 and *H. pylori* gastritis. The most significant species are identified at the top.
620 Differential expression analysis based on the Negative Binomial (Gamma-Poisson)
621 distribution. Streptococcus identified by BLAST as S. mitis with 98% coverage, 99%
622 identity and Neisseria mucosa had 98% coverage and 100% identity. None of these
623 OTUs remained significant when *H. pylori* was removed from the analysis.

624 **Table S4B**. Significant bacterial genera identified between autoimmune atrophic
625 gastritis and *H. pylori*-induced atrophic gastritis. The most significant species are
626 identified at the top. Differential expression analysis based on the Negative Binomial
627 (Gamma-Poisson) distribution. NB when *H. pylori* was removed from the analysis
628 these genera remained significant, with an additional genus *Desulfobulbus* also
629 reaching significance.

630
631
632
633
634

635 **REFERENCES**

636 1 Ferlay J, Soerjomataram I, Ervik M, *et al.* GLOBOCAN 2012 v1.0, Cancer
637 Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon,
638 France: International Agency for Research on Cancer; 2013. Available from:
639 http://globocan.iarc.fr, accessed on 19/07/16.
640 2 Uemura N, Okamoto S, Yamamoto S, *et al.* Helicobacter pylori infection and
641 the development of gastric cancer. The New England journal of medicine
642 2001;**345**:784-9.
643 3 Vannella L, Lahner E, Osborn J, *et al*. Systematic review: gastric cancer
644 incidence in pernicious anaemia. Alimentary pharmacology & therapeutics
645 2013;**37**:375-82.
646 4 Burkitt MD, Pritchard DM. Review article: Pathogenesis and management of
647 gastric carcinoid tumours. Alimentary pharmacology & therapeutics 2006;**24**:1305-
648 20.
649 5 Song H, Zhu J, Lu D. Long-term proton pump inhibitor (PPI) use and the
650 development of gastric pre-malignant lesions. The Cochrane database of systematic
651 reviews 2014:CD010623.
652 6 Bik EM, Eckburg PB, Gill SR, *et al.* Molecular analysis of the bacterial
653 microbiota in the human stomach. Proceedings of the National Academy of Sciences
654 of the United States of America 2006;**103**:732-7.
655 7 Huang BR, Tsai CF, Lin HY, *et al.* Interaction of inflammatory and anti-
656 inflammatory responses in microglia by Staphylococcus aureus-derived lipoteichoic
657 acid. Toxicology and applied pharmacology 2013;**269**:43-50.

658 8 Correa P. Human gastric carcinogenesis: a multistep and multifactorial
659 process--First American Cancer Society Award Lecture on Cancer Epidemiology and
660 Prevention. Cancer research 1992;**52**:6735-40.
661 9 Ziebarth D, Spiegelhalder B, Bartsch H. N-nitrosation of medicinal drugs
662 catalysed by bacteria from human saliva and gastro-intestinal tract, including
663 Helicobacter pylori. Carcinogenesis 1997;**18**:383-9.
664 10 Biarc J, Nguyen IS, Pini A, *et al.* Carcinogenic properties of proteins with pro-
665 inflammatory activity from Streptococcus infantarius (formerly S.bovis).
666 Carcinogenesis 2004;**25**:1477-84.
667 11 Brestoff JR, Artis D. Commensal bacteria at the interface of host metabolism
668 and the immune system. Nature immunology 2013;**14**:676-84.
669 12 Catsburg CE, Gago-Dominguez M, Yuan JM, *et al.* Dietary sources of N-
670 nitroso compounds and bladder cancer risk: Findings from the Los Angeles bladder
671 cancer study. International journal of cancer Journal international du cancer 2013.
672 13 Keszei AP, Goldbohm RA, Schouten LJ, *et al*. Dietary N-nitroso compounds,
673 endogenous nitrosation, and the risk of esophageal and gastric cancer subtypes in
674 the Netherlands Cohort Study. The American journal of clinical nutrition
675 2013;**97**:135-46.
676 14 Kumar JD, Steele I, Moore AR, *et al.* Gastrin stimulates MMP-1 expression in
677 gastric epithelial cells: putative role in gastric epithelial cell migration. American
678 journal of physiology Gastrointestinal and liver physiology 2015;**309**:G78-86.
679 15 Moore AR, Boyce M, Steele IA, *et al*. Netazepide, a gastrin receptor
680 antagonist, normalises tumour biomarkers and causes regression of type 1 gastric
681 neuroendocrine tumours in a nonrandomised trial of patients with chronic atrophic
682 gastritis. PloS one 2013;**8**:e76462.

16 Boyce M, Moore AR, Sagatun L, *et al.* Netazepide, a gastrin/cholecystokinin-2 receptor antagonist, can eradicate gastric neuroendocrine tumours in patients with autoimmune chronic atrophic gastritis. Br J Clin Pharmacol 2016.

17 TRI-Reagent®. Extraction Protocol. http://www.sigmaaldrich.com/technical-documents/protocols/biology/tri-reagent.html Accessed 16/12/15.

18 Dockray GJ. Immunochemical studies on big gastrin using NH2-terminal specific antisera. Regulatory peptides 1980;**1**:169-86.

19 Varro A, Ardill JE. Gastrin: an analytical review. Annals of clinical biochemistry 2003;**40**:472-80.

20 Laufer AS, Metlay JP, Gent JF, *et al*. Microbial communities of the upper respiratory tract and otitis media in children. mBio 2011;**2**:e00245-10.

21 Salter SJ, Cox MJ, Turek EM, *et al.* Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC biology 2014;**12**:87.

22 Manichanh C, Rigottier-Gois L, Bonnaud E, *et al.* Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut 2006;**55**:205-11.

23 Li TH, Qin Y, Sham PC, *et al*. Alterations in Gastric Microbiota After H. Pylori Eradication and in Different Histological Stages of Gastric Carcinogenesis. Scientific reports 2017;**7**:44935.

24 Gao Z, Guo B, Gao R, *et al*. Microbiota disbiosis is associated with colorectal cancer. Front Microbiol 2015;**6**:20.

25 Nakae H, Tsuda A, Matsuoka T, *et al*. Gastric microbiota in the functional dyspepsia patients treated with probiotic yogurt. BMJ Open Gastroenterol 2016;**3**:e000109.

26 von Rosenvinge EC, Song Y, White JR, *et al*. Immune status, antibiotic medication and pH are associated with changes in the stomach fluid microbiota. The ISME journal 2013;**7**:1354-66.

27 Paroni Sterbini F, Palladini A, Masucci L, *et al.* Effects of Proton Pump Inhibitors on the Gastric Mucosa-Associated Microbiota in Dyspeptic Patients. Applied and environmental microbiology 2016;**82**:6633-44.

28 Jackson MA, Goodrich JK, Maxan ME, *et al.* Proton pump inhibitors alter the composition of the gut microbiota. Gut 2016;**65**:749-56.

29 Schulz C, Schutte K, Koch N, *et al.* The active bacterial assemblages of the upper GI tract in individuals with and without Helicobacter infection. Gut 2016.

30 Martin ME, Bhatnagar S, George MD, *et al.* The impact of Helicobacter pylori infection on the gastric microbiota of the rhesus macaque. PloS one 2013;**8**:e76375.

31 Eun CS, Kim BK, Han DS, *et al.* Differences in Gastric Mucosal Microbiota Profiling in Patients with Chronic Gastritis, Intestinal Metaplasia, and Gastric Cancer Using Pyrosequencing Methods. Helicobacter 2014;**19**:407-16.

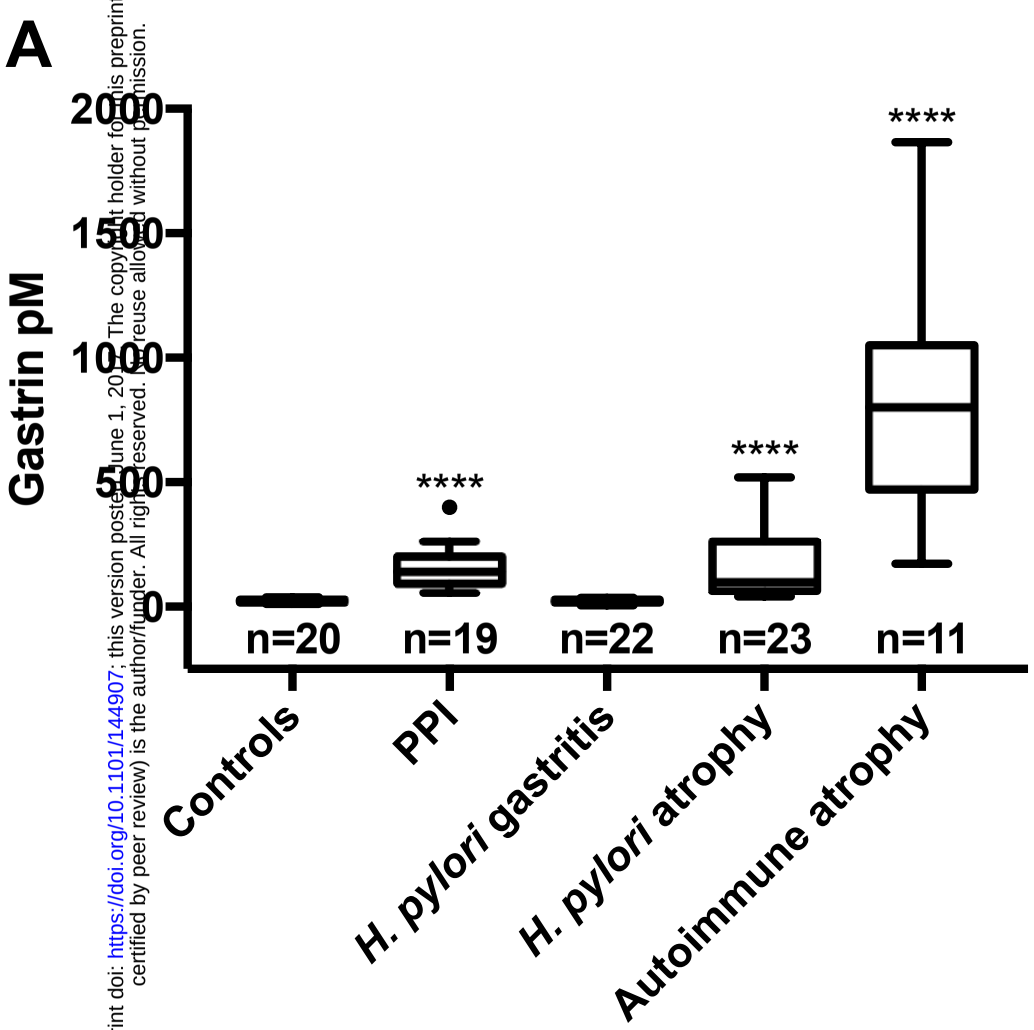
32 Farrell JJ, Zhang L, Zhou H, *et al.* Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer. Gut 2012;**61**:582-8.

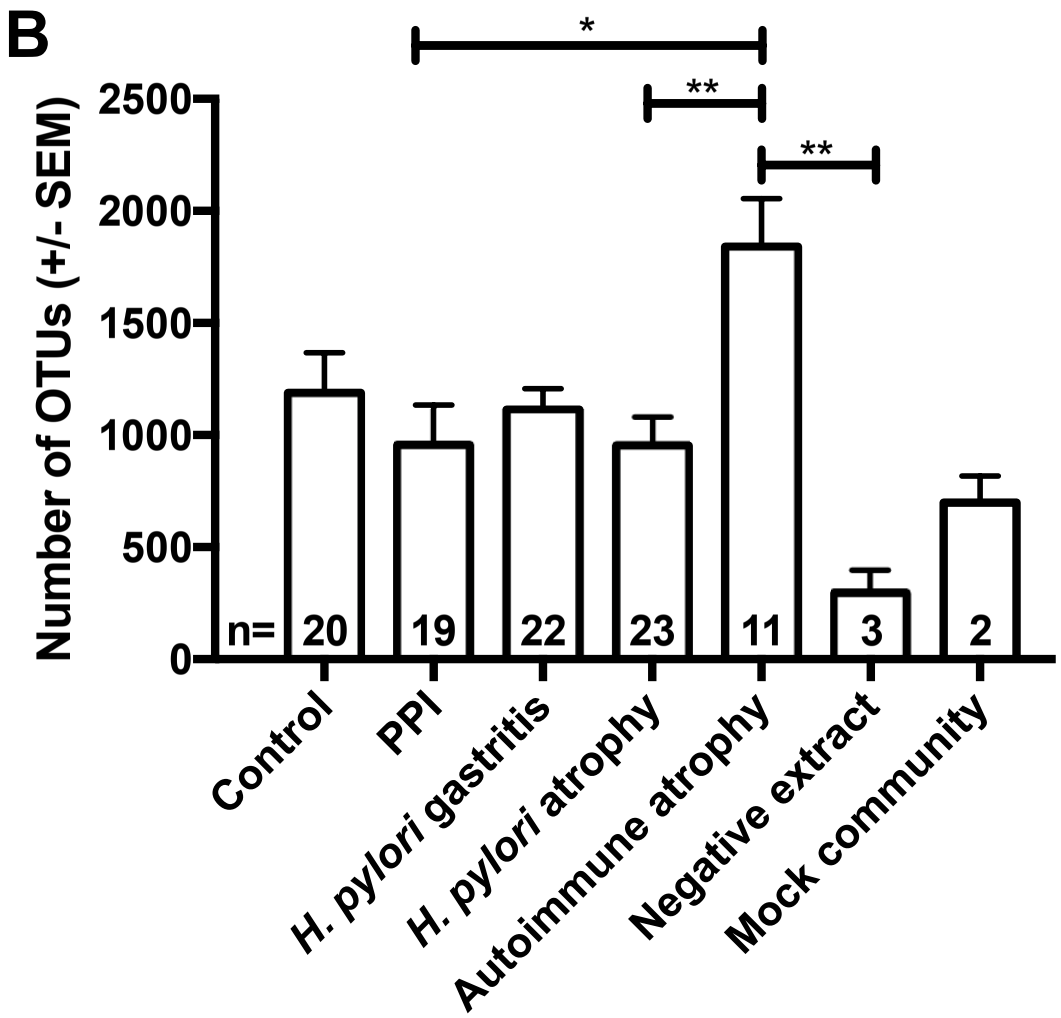
33 Muto M, Hitomi Y, Ohtsu A, *et al.* Acetaldehyde production by non-pathogenic Neisseria in human oral microflora: implications for carcinogenesis in upper aerodigestive tract. International journal of cancer Journal international du cancer 2000;**88**:342-50.

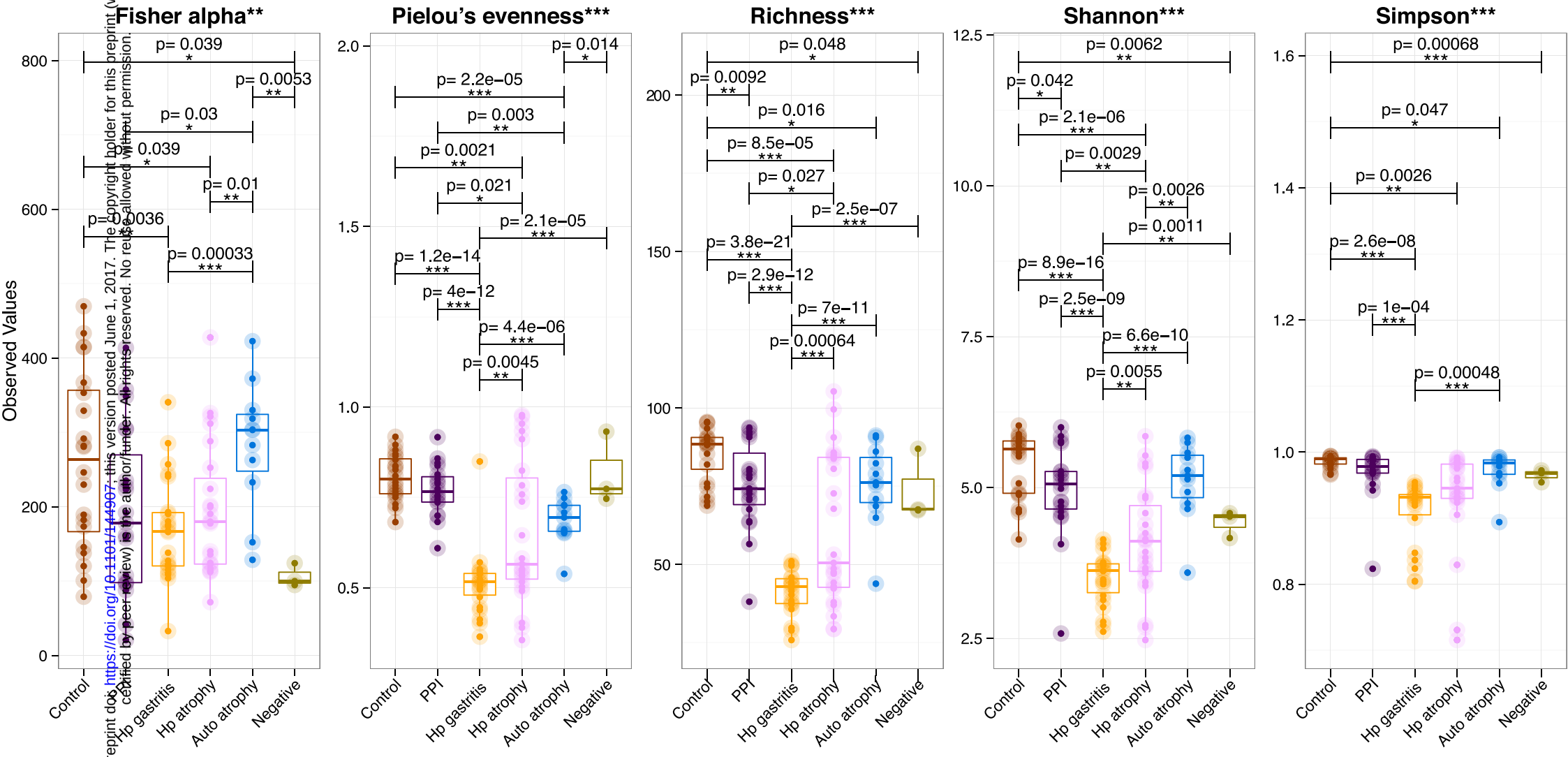
34 Khosravi Y, Dieye Y, Loke MF, *et al*. Streptococcus mitis Induces Conversion of Helicobacter pylori to Coccoid Cells during Co-Culture In Vitro. PloS one 2014;**9**:e112214.

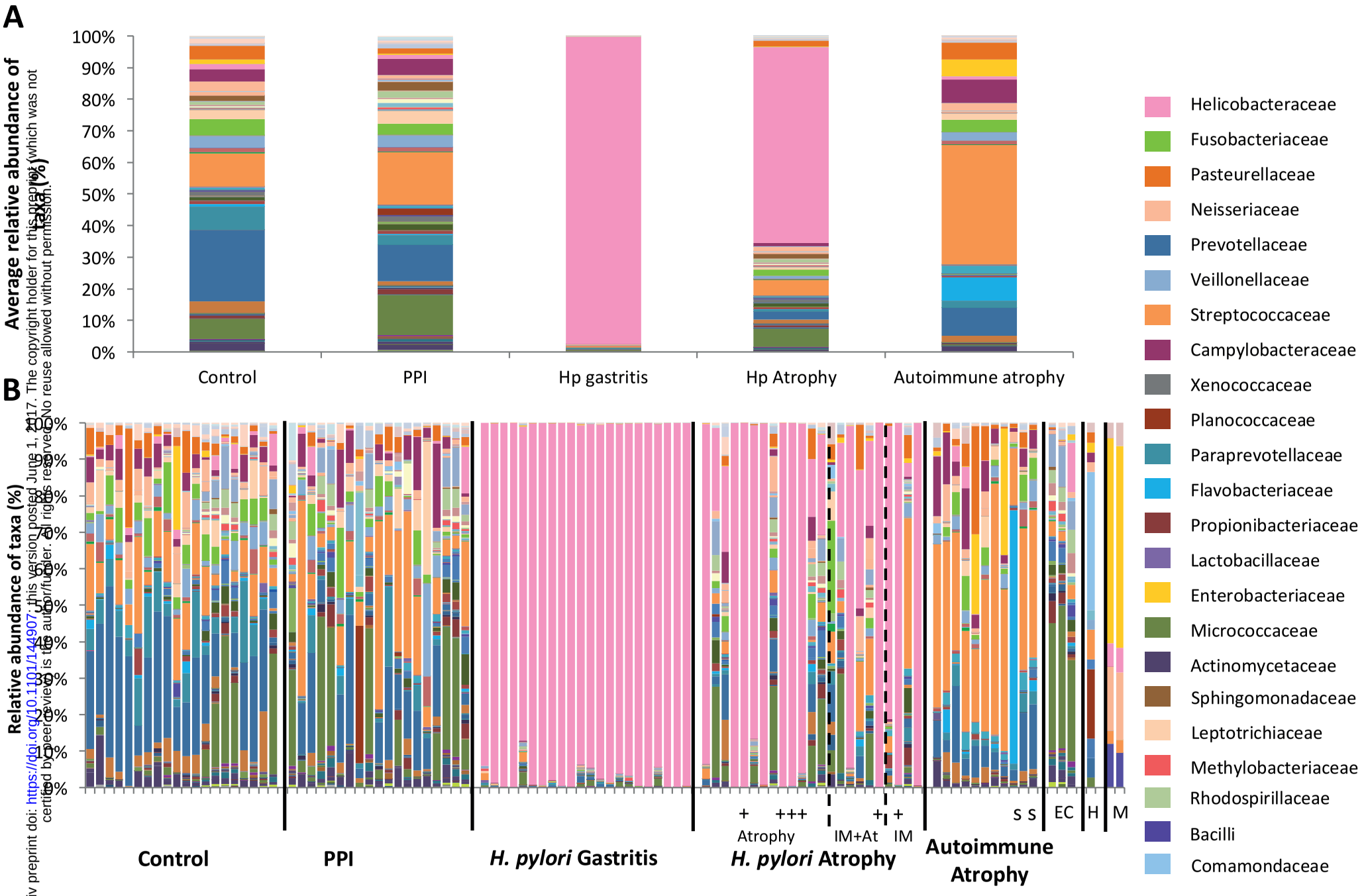
732 35 Chan WY, Hui PK, Leung KM, *et al*. Coccoid forms of Helicobacter pylori in
733 the human stomach. American journal of clinical pathology 1994;**102**:503-7.
734 36 Li N, Han L, Chen J, *et al*. Proliferative and apoptotic effects of gastric
735 epithelial cells induced by coccoid Helicobacter pylori. Journal of basic microbiology
736 2013;**53**:147-55.
737 37 Kim HS, Lee HE, Yang HK, *et al*. High lactate dehydrogenase 5 expression
738 correlates with high tumoral and stromal vascular endothelial growth factor
739 expression in gastric cancer. Pathobiology 2014;**81**:78-85.
740 38 Wang J, Yuan W, Chen Z, *et al.* Overexpression of G6PD is associated with
741 poor clinical outcome in gastric cancer. Tumour Biol 2012;**33**:95-101.
742 39 Lancaster CR, Kroger A, Auer M, *et al*. Structure of fumarate reductase from
743 Wolinella succinogenes at 2.2 A resolution. Nature 1999;**402**:377-85.
744 40 Ge Z. Potential of fumarate reductase as a novel therapeutic target in
745 Helicobacter pylori infection. Expert Opin Ther Targets 2002;**6**:135-46.
746 41 Kassem, II, Khatri M, Sanad YM, *et al.* The impairment of
747 methylmenaquinol:fumarate reductase affects hydrogen peroxide susceptibility and
748 accumulation in Campylobacter jejuni. Microbiologyopen 2014;**3**:168-81.
749 42 Wang YM, Gu ML, Ji F. Succinate dehydrogenase-deficient gastrointestinal
750 stromal tumors. World journal of gastroenterology : WJG 2015;**21**:2303-14.
751 43 Coulton GR, Firth JA. Cytochemical evidence for functional zonation of
752 parietal cells within the gastric glands of the mouse. Histochem J 1983;**15**:1141-50.

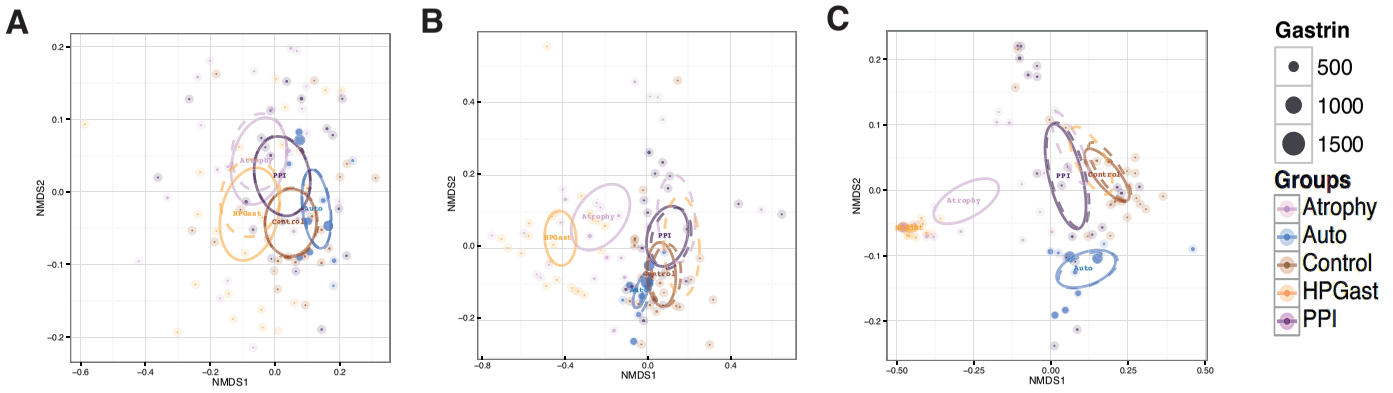
753





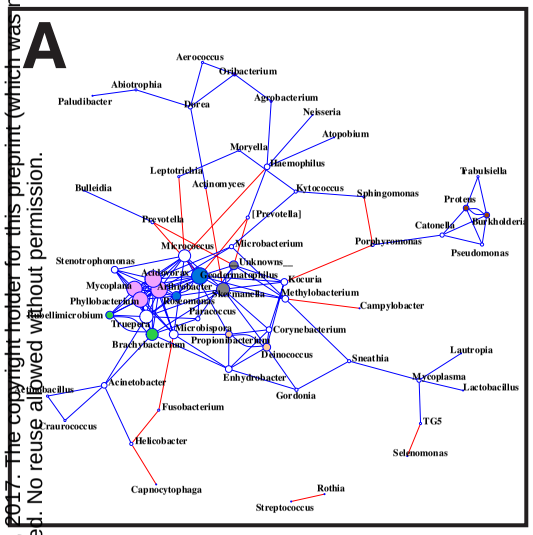




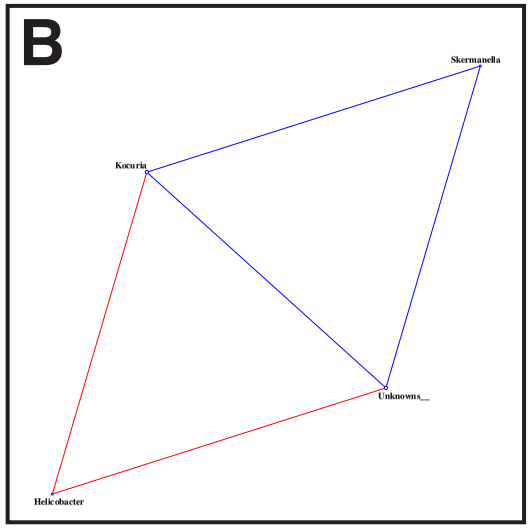


D

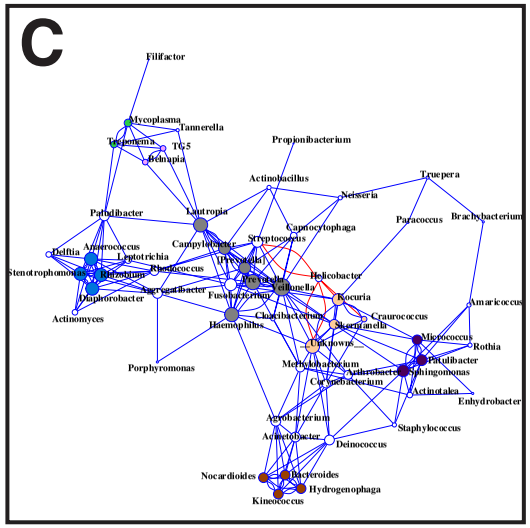
		H. pylori included in analysis			*H. pylori* removed from analysis		
Group Comparison		**Unifrac significance (presence/absence)**	**Bray-Curtis significance (abundance)**	**Weighted Unifrac (abundance)**	**Unifrac significance (presence/absence)**	**Bray-Curtis significance (abundance)**	**Weighted Unifrac (abundance)**
Control	PPI	ns	ns	0.0116 *	ns	ns	0.0132 *
	Hp Gastritis	ns	0.0270 *	1.3741×10^{-12} ***	ns	ns	ns
	Hp Atrophy	0.059	ns	ns	ns	0.0193*	ns
	Autoimmune	0.0638	ns	ns	0.0564	ns	ns
PPI	Hp Gastritis	ns	0.0008 ***	9.9989×10^{-25} ***	ns	ns	ns
	Hp Atrophy	ns	ns	ns	ns	ns	ns
	Autoimmune	0.0019 **	0.004 **	0.0037 **	0.0018 **	0.0043 **	0.0034 **
Hp Gastritis	Hp Atrophy	ns	0.0080 **	4.1694×10^{-16} ***	ns	ns	ns
	Autoimmune	0.0060 **	ns	2.5084×10^{-9} ***	0.0090 **	0.0249*	0.0233*
Hp Atrophy	Autoimmune	3.159×10^{-5} ***	ns	0.0568	1.191×10^{-4} ***	3.765×10^{-4} ***	0.0227*



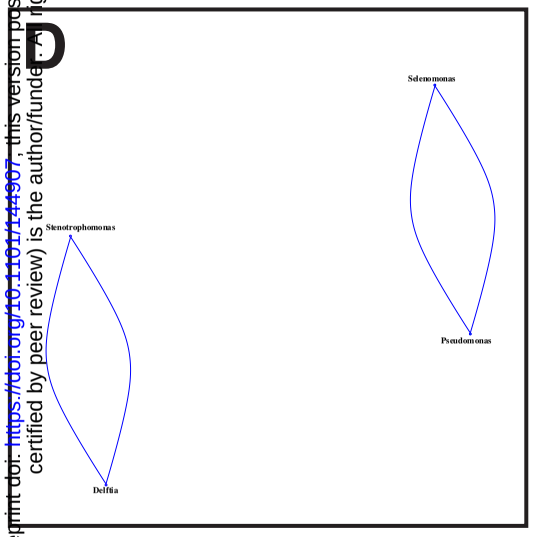
Normal



H. pylori gastritis



H. pylori atrophy



Autoimmune atrophy