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Complete List of Authors:	<p>Forbes, Jessica; University of Manitoba, Medical Microbiology and Infectious Diseases; National Microbiology Laboratory, Public Health Agency of Canada</p> <p>Van Domselaar, Gary; University of Manitoba, Medical Microbiology and Infectious Diseases; National Microbiology Laboratory, Public Health Agency of Canada</p> <p>Sargent, Michael; Department of Internal Medicine and the University of Manitoba IBD Clinical and Research Centre</p> <p>Green, Chris; University of Manitoba, Department of Community Health Sciences</p> <p>Springthorpe, Susan; University of Ottawa, Centre for Research on Environmental Microbiology</p> <p>Krause, Denis; University of Manitoba, Medical Microbiology and Infectious Diseases</p> <p>Bernstein, Charles; Department of Internal Medicine and the University of Manitoba IBD Clinical and Research Centre</p>
Keyword:	water microbiome, inflammatory bowel disease, epidemiology, 16s rDNA, etiology

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Microbiome Profiling of Drinking Water in Relation to Incidence of Inflammatory Bowel Disease

Jessica D. Forbes^{1,2}, Gary Van Domselaar^{1,2}, Michael Sargent³, Chris Green⁴, Susan Springthorpe⁵,
Denis O. Krause^{1,†}, Charles N. Bernstein^{3,*}

Author Affiliations

¹ Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Canada

² National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada

³ Department of Internal Medicine and the University of Manitoba IBD Clinical and Research Centre,
Winnipeg, Manitoba, Canada

⁴ Department of Community Health Sciences, Faculty of Medicine, University of Manitoba, Canada

⁵ Centre for Research on Environmental Microbiology, University of Ottawa, Ottawa, ON

[†]Deceased 16 October 2011.

*Corresponding author: Charles N. Bernstein Charles.Bernstein@umanitoba.ca, 804F-715 McDermot
Avenue, Winnipeg, Manitoba, Canada R3E3P4, Phone: (204) 789-3369, Fax: (204) 789-3972

Jessica D. Forbes umforb25@myumanitoba.ca;

Michael Sargent Michael.Sargent@umanitoba.ca;

Gary Van Domselaar Gary.VanDomselaar@phac-aspc.gc.ca;

Chris Green chris.green.wpg@gmail.com;

Susan Springthorpe spring@uottawa.ca

ABSTRACT

The etiology of IBD is unknown; current research is focused on determining environmental factors. One consideration is drinking water: water systems harbour a considerable diversity of microbes with bacterial concentrations estimated at 10^6 - 10^8 cells per liter. Perhaps differences in microbial ecology of water sources may impact on differential incidence rates of IBD. Regions of Manitoba were geographically mapped according to incidence rates of IBD and identified as high (HIA) or low (LIA) incidence areas. Bulk water, filter material and pipe wall samples were collected from public buildings in different jurisdictions and their population structure analyzed using 16S rDNA sequencing. At the phylum level Proteobacteria were observed significantly less frequently ($P=0.02$) in HIA versus LIA. The abundance of Proteobacteria was also found to vary according to water treatment distribution networks. Gammaproteobacteria was the most abundant class of bacteria and was observed more frequently ($P=0.006$) in LIA. At the genus level, microbes found to associate with HIA include *Bradyrhizobium* ($P=0.02$) and *Pseudomonas* ($P=0.02$). Particular microbes were found to associate with LIA or HIA, based on sample location and/or type. This work lays out a basis for further studies exploring water as a potential environmental source for IBD triggers.

Keywords

Inflammatory bowel disease, 16S rDNA, water microbiome, etiology, epidemiology

List of Abbreviations

IBD – inflammatory bowel disease; HIA – high incidence area; LIA – low incidence area; UMIBDED – University of Manitoba IBD Epidemiology Database; CD – Crohn's disease; UC – ulcerative colitis; OTU – operational taxonomic unit

44

45 **Competing Interests**

46 Dr Bernstein is supported in part by the Bingham Chair in Gastroenterology. In the past year he has
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Draft

INTRODUCTION

Canada has among the highest reported incidence rates of inflammatory bowel disease (IBD) worldwide (Molodecky et al. 2012). There are an estimated 250,000 Canadians suffering from IBD (<http://cdhf.ca>). The first population based incidence rates from Canada were reported from the province of Manitoba, a central province with a population of 1.27 million in 2013 (Bernstein et al. 1999). Capitalizing on universal health care provided within each province and the administrative health databases established within each province, investigators have shown that these high rates in Manitoba are comparably elevated in at least 5 of the other 9 provinces (Bitton et al. 2014; Bernstein et al. 2006).

IBD is an idiopathic, multifaceted disease linked to genetic, immunological and notably, environmental elements. Possible environmental contributions include diet (Vagianos et al. 2016), stress (Iglesias-Rey et al. 2014), medications (Osterman et al. 2014) and smoking (Parkes et al. 2014). A number of factors appear to play a role in influencing the clinical course of disease, however, there is a paucity of data establishing a clear cause-and-effect relationship with IBD.

Many acute illnesses may be secondary to the consumption of contaminated drinking water including legionellosis (Cohn et al. 2014), cholera (Alam et al. 2014) and gastroenteritis (Beaudeau et al. 2014). It is unknown if immunocompetent hosts can acquire chronic diseases like IBD through microbes present in drinking water. Linkages between the development of IBD and specific microorganisms have been investigated such as *Mycobacterium paratuberculosis* (Rhodes et al. 2013; Suwandi et al. 2014) or adherent invasive *Escherichia coli* (Kotlowski et al. 2007; Agus et al. 2014). In the past decade studies have focused on exploring the gut microbiome using molecular techniques for microorganisms

73 that may either be protective or injurious to the healthy gut and associated with one form of IBD or
 74 another (Balzola et al. 2011; Frank et al. 2007).

75

76 While it is theoretically compelling that any or all components of the environment could be involved
 77 in pathogen exposure, one realistic and universal geographically-based component is drinking water.
 78 The average Canadian adult consumes 1.5 litres of water daily (<http://www.hc-sc.gc.ca>). Studies have
 79 suggested a risk of developing IBD to be associated with iron content of drinking water (Aamodt et al.
 80 2008) as iron may impact the microbial ecology of the human gut (Dostal et al. 2012). However, the
 81 concept of the drinking water microbiome as an etiological source of IBD is novel and may provide
 82 rationale to the varied geographical distribution of incidence rates observed globally. Drinking water
 83 systems harbor a vast diversity of microbes (Hammes et al. 2008; Navarro-Noya et al. 2013); bacterial
 84 concentrations of drinking water are estimated at 10^6 - 10^8 cells per liter (Hammes et al. 2008;
 85 Navarro-Noya et al. 2013; Lautenschlager et al. 2010). Modern water treatment systems apply
 86 filtration and disinfection methods to purify drinking water and inactivate bacteria though it is not
 87 possible to extricate all microbes from drinking water distribution systems due to 1) microbes'
 88 survival and persistence including biofilm formation and nitrification and 2) inadequate disinfection
 89 strategies and a lack of understanding of microbial ecology (Berry et al. 2006). Bacterial communities
 90 of drinking water systems may be innocuous, may benefit human health or may be detrimental if
 91 harbouring potential pathogen (Berry et al. 2006; Thomas and Ashbolt 2011), therefore it is critical to
 92 determine the identity of bacteria present in water distribution systems. Further, microorganisms that
 93 are not considered pathogenic may be injurious in specific hosts whose intestinal immune system
 94 may be genetically programmed to react aberrantly to them.

95

96 The primary goal of this study was to investigate whether the microbial communities of bulk drinking
97 water differs between high (HIA) and low incidence areas (LIA) of IBD and to what extent. The
98 identification of novel microbes widely present in HIA while absent or less frequent in LIA, or
99 alternatively, microbes present in LIA that are absent in HIA may aid in understanding of the etiology
100 of IBD. Secondly, we explored the microbial populations of different sample material including bulk
101 drinking water, filters, and pipe wall (biofilm). Lastly, we assessed microbial communities among a
102 number of sample locations and their associated water distribution system. Herein, we perform a
103 community structure analysis of a number of aspects of the water microbiota to provide comparative
104 associations between microbial community composition and abundance. To our knowledge this work
105 represents the first study investigating the link between the microbiota of drinking water and its
106 association with IBD.

107 **MATERIALS AND METHODS**

108 *Water supply*

109 The city of Winnipeg receives its water from Shoal Lake, a large isolated lake in southeastern
110 Manitoba (<http://winnipeg.ca/waterandwaste/>). It is 137 kilometers from Winnipeg and
111 approximately 92 metres higher. Water flows via gravity through the aqueduct to Deacon Reservoir, a
112 four-cell open reservoir on the eastern side of Winnipeg. The City of Winnipeg’s water treatment
113 process includes dissolved air flotation, ozonation, filtration and ultraviolet light disinfection. Fluoride
114 and orthophosphate are added then water flows from the water treatment plant to one of three
115 regional reservoirs and pumping stations. Chlorine is added and water flows through the distribution
116 system. Brandon, Manitoba a city of approximately 50,000 is 199 kilometers west of Winnipeg, uses
117 water from the Assiniboine River as a source for its treatment plant; Steinbach, Manitoba, a city of
118 approximately 14,000 is 58 kilometers southeast of Winnipeg uses groundwater as its drinking source.
119 Both rural communities employ water disinfection strategies similar to those just described; however,
120 the key difference is the source of the drinking water.

121
122 Public buildings were used as sample locations as they were a source of high consumption of water.
123 Verbal permission was obtained from personnel at each location. Each location was equipped with a
124 water filtration system and three pieces of PVC piping. The PVC piping was used to collect biofilm that
125 naturally occurs in drinking water pipes. The water filters were changed every 3 months and the PVC
126 replaced every 4 months over the course of 1 year, providing replicates for each sampling location.
127 Samples were collected in 2005 from HIA (n=20) and LIA (n=20) across Winnipeg, Brandon and
128 Steinbach. We collected samples from 3 water distribution networks (Shoal Lake (n=12), Brandon
129 (n=4) and Steinbach (n=4)). Of the water distributed to Winnipeg areas, samples were obtained from

5 reservoirs – Shoal Lake Intake (n=3), Deacon (n=3), McLean (n=5), McPhillips (n=14) and Wilkes (n=7). Sample types used in this analysis include, bulk drinking water (n=8), filter (n= 15) and pipe wall material (n=17). Sample meta-data can be found in Supplementary Table 1. Samples were removed from the installed PVC system one section at a time. They were placed in zip locked sealed bags, shipped to the laboratory and stored at 4°C until processing.

Data sources and sample collection

Our group previously identified HIA and LIA using the University of Manitoba IBD Epidemiology Database (UMIBDED; Figure 1) (Green et al. 2006); the incidence of HIA was ≥ 2 -fold when compared to LIA. The UMIBDED is a population-based archive dating back to 1984 as described in detail elsewhere (Bernstein et al. 2006); it was created from the Manitoba Health databases and includes all Manitobans who meet a validated administrative case definition for IBD.

Incidence rates were calculated for 2001 for the purpose of this study. We have reassessed small area incidence rates for 2009-2010 and they are minimally changed from 2001 (data not shown). Hence, we anticipate HIA and LIA remained as such in 2005 when water samples were collected. It is worth noting that based on epidemiological surveillance, LIA of Crohn's disease (CD) correlate highly with LIA of ulcerative colitis (UC), with a similar trend observed in HIA.

DNA extraction

The following nucleic acid extraction protocol was implemented for optimal DNA recovery as the biomass of water was expected to be low: 1 ml of each sample was inoculated in 9 ml 2.5% LB broth and incubated aerobically at 37°C until slightly turbid. The culture suspension was used for genomic

153 DNA extraction with Zymo Research Fecal DNA Kits. The DNA concentration and purity were assessed
154 by microspectrophotometry (Beckman DU/800; Beckman Coulter, Inc., Fullerton, CA). Genomic DNA
155 samples were diluted to a concentration of 20 ng/μl.

156

157 *Sequencing*

158 The bacterial 16S rRNA genes were amplified with primers 28F (5'-GAGTTTGATCCTGGCTCAG-3') and
159 519R (5'-GTNTTACNGCGGCKGCTG-3') targeting the V1-V3 hypervariable region (approximately 510
160 bp). Pyrosequencing was conducted at Research and Testing Laboratory (Lubbock, TX;
161 <http://Researchandtesting.com>) using a 454 GS FLX (454 Life Sciences, a Roche Company, Brandford,
162 CT) and titanium technology. Sequencing generated 110,760 raw reads.

163

164 *Comparative sequence analysis*

165 Quality control and taxonomic profiling was conducted using mothur (v.1.34.0) (Schloss et al. 2009), a
166 microbial community software program. Barcodes and primers were removed and low quality reads
167 were filtered based on an average quality score <20, having read lengths <210 bp, containing
168 homopolymers >8 nucleotides and containing any ambiguous base calls. Reads were aligned against
169 the 16S rDNA SILVA database (Pruesse et al. 2007). Sequencing noise was reduced by clustering reads
170 that differ by only 1 bp. Chimeric sequences were detected using mothur's implementation of
171 UCHIME and removed (Edgar et al. 2011). The remaining reads were binned into species-level (≥97%
172 sequence similarity) operational taxonomic units (OTUs) using the average neighbour algorithm, and
173 taxonomic classification was performed using the SILVA database with Ribosomal Database Project
174 taxonomy and a 70% minimum bootstrap (Wang et al. 2007).

175

176 *Alpha diversity*

177 Species richness estimates and diversity indices were calculated by algorithms implemented in
178 mothur (Schloss et al. 2009).

179

180 *Statistical analysis*

181 Analyses were executed using the R package (<http://www.r-project.org>) phyloseq (McMurdie and
182 Holmes 2013). Samples with <500 reads were removed. OTUs were normalized to relative abundance
183 and filtered to include only OTUs with a mean abundance of $>10^{-4}$ across all samples. Statistical
184 significance of community composition differences was tested using customized R-scripts: the
185 Kruskal-Wallis one-way analysis of variance was applied to microbiota data and community estimates
186 to compare similarities. *P*-values were considered significant at $P < 0.05$. Visualization of OTU-based
187 community composition differences was employed using the Bray-Curtis dissimilarity index.

RESULTS AND DISCUSSION

Bacterial Community Characterization

Filtering out low quality, chimeric and non-bacterial reads generated 83,392 high quality 16S rDNA reads with an average amplicon length of 456 bp. The sequencing depth was comparable among groups with 2323 ± 770 in HIA and 2253 ± 693 in LIA. Therefore, with an average of 2287 reads per sample we achieved a fairly deep sequencing depth capturing the majority of bacterial OTUs. Sequences were clustered into 749 OTUs based on their shared sequence similarity at a 97% threshold (3% sequence divergence). Four samples were excluded from statistical analysis due to insufficient read numbers (<500).

The average coverage for OTU characterization based on Good's coverage was 99.1% (min-90.0%; max-99.8%). Richness (Chao1 and ACE) and diversity (Shannon and Simpson) were comparable among incidence areas (Figure 2a) and sample type (Figure 2b). Investigation of the 7 sampling locations identified differences of richness as estimated by ACE ($P=0.046$; Figure 2c). To further investigate richness estimates and diversity indices, sample locations were divided into three respective water distribution systems: all Winnipeg locations receiving its water from Shoal lake, Brandon (Assiniboine river) and Steinbach (ground-water). Both Chao ($P=0.04$) and ACE ($P=0.01$) richness estimators indicate species richness to vary among the different water systems (Figure 2d). For example, species richness was highest in Steinbach followed by Winnipeg (Shoal Lake) and Brandon. Previous studies suggest that the use of chlorination in water treatment processes significantly reduces the number of observed species (Roeselers et al. 2015); chlorination is used in all water treatment distribution systems presented in this study. In terms of IBD incidence, species richness was lowest in Brandon

and highest in Steinbach which were selected communities of HIA and LIA, respectively. It's possible that the use and associated levels of chlorine could have some effect on incidence areas.

Bacterial population comparisons

Bacterial communities of LIA and HIA

Sequences were assigned to 10 phyla, of which phylotypes belonging to the Proteobacteria dominated the overall microbial population. Proteobacteria as a principal phylum in drinking water is well documented in the literature (Lautenschlager et al. 2014; Wang et al. 2014; Liu et al. 2014; Lührig et al. 2015). The average relative abundance of Proteobacteria was 92%. This corresponds with related population-based studies whereby the abundance of Proteobacteria can range anywhere from 44 to 98% (Wang et al. 2014; Liu et al. 2014; Lührig et al. 2015). The abundance of Proteobacteria was higher in LIA ($94\% \pm 21$) in contrast to HIA ($91\% \pm 22$; $P=0.02$; Figure 3a, Supplementary Table 2). Furthermore, while there was a lack of significant variation of Proteobacteria between sample locations (Supplementary Table 3), there was a significant distinction in abundance between water treatment systems ($P=0.046$; Steinbach (100%) > Shoal Lake ($93\% \pm 20$) > Brandon ($76\% \pm 41$); Supplementary Table 4). Actinobacteria, Firmicutes and Bacteroidetes also represent common phyla. Phyla present at very low amounts included Acidobacteria, Chloroflexi, Fusobacteria, Spirochaetes, TM7 and Verrucomicrobia.

In our analysis, Gammaproteobacteria showed an overall dominance of 54%. This is intriguing, as many population-based or environmental studies have reported either Beta- (Pinto et al. 2012; Lin et al. 2014) or Alphaproteobacteria (Gomez-Alvarez et al. 2012; Bai et al. 2013) to outweigh the Gammaproteobacteria population. The abundance of Gammaproteobacteria was found to differ

233 between IBD incidence areas ($P=0.006$). Specifically, Gammaproteobacteria was elevated in LIA (77%
234 ± 41) when compared to HIA ($28\% \pm 45$). We observed moderate changes of Alphaproteobacteria
235 ($P=0.07$), which were highest in LIA. Investigation of sample type presented an interesting dynamic:
236 the abundance of Alphaproteobacteria ($P=0.055$) and Betaproteobacteria ($P=0.091$) seemed to shift
237 based on the sample type (Supplementary Table 5). A noteworthy observation from this study is that
238 these microbial populations are inversely correlated, for example, Alphaproteobacteria (competitive
239 under oligotrophic conditions and degrading complex organic compounds) are more prevalent within
240 pipe walls whereas Betaproteobacteria (fast-grower and nutrient lovers) (Newton et al. 2011) are
241 more prevalent within water and filter material. Rudi et al. (2010) observed a similar phenomenon
242 utilizing a ProteoQuant assay; the authors concluded that the Alpha- and Betaproteobacteria are in
243 competition, independent of the abundance of Gammaproteobacteria. A moderate variation of
244 Betaproteobacteria was observed among sample locations ($P=0.056$).
245
246 Few genera were found to correlate with either incidence area; *Pseudomonas*
247 (Gammaproteobacteria; $P=0.016$) was highest in LIA whereas *Bradyrhizobium* (Alphaproteobacteria;
248 $P=0.02$) was highest in HIA (Figure 4a). *Pseudomonas* comprised approximately 48% of the microbial
249 population. Such a predominance of *Pseudomonas* is of particular concern as many species belonging
250 to this genus are biofilm formers (Fazli et al. 2014), which therefore lead to an increase in pathogen
251 persistence. *Pseudomonas* has been implicated in the pathogenesis of IBD; the *Pseudomonas*
252 *fluorescens*-associated sequence I2 that encodes for a T cell superantigen is prevalent among CD (38-
253 60%) and UC (42%) (Prideaux et al. 2012; Wei et al. 2002). Huang et al. (2014) reported *Pseudomonas*
254 *aeruginosa* to represent 11.92% of total sequence reads of filtered water and 11.16% in drinking
255 water; hence, *Pseudomonas* was found to be resistant to particular disinfection methods due to

256 unknown mechanisms. Indeed, many studies support these findings in that *P. aeruginosa* is frequently
257 detected in chlorinated drinking water (Lee et al. 2011; Wingender and Flemming 2011). Conversely,
258 *Bradyrhizobium* has previously been identified to be a dominant member of chlorinated water
259 (Gomez-Alvarez et al. 2012). The opportunistic pathogenic association, if any, of *Bradyrhizobium*
260 species to human gastrointestinal diseases is not well defined. To our knowledge, *Bradyrhizobium* has
261 not been implicated in the pathogenesis of IBD; however, *Bradyrhizobium enterica* is a newly
262 identified bacterium and has been identified among persons with cord colitis following umbilical-cord
263 hematopoietic stem-cell transplantation (Bhatt et al. 2013). It should be mentioned that both
264 *Pseudomonas* and *Bradyrhizobium* have been reported to be a common contaminant of next
265 generation sequencing studies, particularly in samples containing low biomass (Salter et al. 2014). To
266 a minor extent, the abundance of *Mycobacterium* ($P=0.06$) was higher in HIA. This genus, particularly,
267 *M. paratuberculosis* is scrutinized in the possible etiology of CD as there are many conflicting reports
268 regarding its relationship to CD (Suwandi et al. 2014, Nazareth et al. 2015). We identified
269 *Mycobacterium* in only 3 samples, all of which were HIA and recovered from pipe wall material.
270 Though many of the taxa identified in our study do not reach statistical significance with respect to
271 abundance, application of a principal coordinate analysis (Figure 5a) shows that many HIA samples
272 cluster separately from LIA thereby suggesting that the overall microbial population structure varies.
273 The HIA samples form a tighter cluster compared to LIA samples; many HIA samples were comprised
274 of a relatively low number of different OTUs reflecting a more homeogenous microbiota. Conversely,
275 LIA were more heterogeneous typically including several different OTUs.

276

277 *Bacterial communities of bulk water*

278 In bulk water, Proteobacteria represented 81% of OTUs, Bacteroidetes represented 10% and
279 Actinobacteria accounted for 9% ($P=0.05$; Figure 3b, Supplementary Table 5). Dominant genera
280 (Figure 4b) of bulk water included *Pseudomonas* (49%), *Janthinobacterium* (18%) and *Massilia* (13%).
281 Liu et al. (2014) has previously characterized the bulk water microbiome; they reported *Polaromonas*
282 (69%), *Sphingomonas* (13%), *Acidovorax* (5%) and *Janthinobacterium* (4%) to dominate the bulk water
283 microbiota. In our data, *Polaromonas*, *Sphingomonas* and *Acidovorax* were identified, albeit at very
284 low abundances. Navarro-Noya et al. (2013) investigated the bacterial communities of drinking water
285 wells in Mexico and concluded similar findings with respect to the relative observed abundance of
286 *Pseudomonas*, *Janthinobacterium* and *Massilia*. Interestingly, they also reported *Perlucidibaca*
287 (13.9%), *Rheinheimera* (5.9%), *Alkanindiges* (2.2%) and *Psychrobacter* (2.1%) to be dominant
288 members of the Gammaproteobacteria. These genera were not observed in our study; we speculate
289 that environmental characteristics such as temperature, in addition to varied water treatment
290 processes explain their absence. Moreover, while *Duganella* and *Flavobacterium* were present in our
291 samples, their abundances were less than previously reported (Navarro-Noya et al. 2013).

292

293 *Bacterial communities of pipe wall (biofilm)*

294 In terms of pipe wall (biofilm), Proteobacteria accounted for 90% of OTUs, and Actinobacteria and
295 Firmicutes represented 5% of OTUs individually (Supplementary Table 5). Within pipe material,
296 *Pseudomonas* (31%), *Sphingomonas* (17%) and *Methylobacterium* (15%) dominated the microbiota.
297 This supplements previous studies (Liu et al. 2014), which have reported a dominance of
298 *Pseudomonas*, *Sphingomonas* and *Janthinobacterium*. Furthermore, Wang et al. (2014) detected
299 *Legionella*, *Mycobacterium* and *P. aeruginosa* in biofilms of pipe material. While our pipe material
300 samples were devoid of *Legionella*, the presence (however paltry) of *Mycobacterium* and

301 *Pseudomonas* is promising with respect to their known potential pathogenesis. The identification and
302 dominance of *Pseudomonas* and *Sphingomonas* detected in pipe wall biofilms has been previously
303 established (Berry et al. 2006; Simões et al. 2010). Both genera are able to form biofilms allowing
304 persistence in otherwise harsh, oligotrophic (*Sphingomonas*) conditions. Moreover, *Sphingomonas*
305 are able to transform between biofilm and planktonic modes that further supports their dominance in
306 bulk water environments (Bereschenko et al. 2010).

307

308 *Bacterial communities of filter material*

309 Proteobacteria represented 99.7% of OTUs associated with filter material (Supplementary Table 5).
310 Intriguingly, Actinobacteria accounted for 9% of OTUs in bulk water yet in filter material their
311 abundance was negligible. A possible explanation for their absence is their planktonic nature and
312 hence do not preferentially attach to surfaces (Newton et al. 2011). *Nitrospirae* are frequently
313 detected in filters but were absent in our samples (Lautenschlager et al. 2014). The main genera of
314 filter material included *Pseudomonas* (67%), *Massilia* (8%) and *Sphingobium* (7%).

315

316 The variation and associated importance of microbial communities between sample types was
317 surprisingly nominal. Previous population-based studies suggest sizeable structural and compositional
318 variability among distinct sample material (Wang et al. 2014; Liu et al. 2014; Lin et al. 2014). In the
319 present study, however, *Acidovorax* ($P=0.06$) was present in both water and filter material whereas
320 *Deefgea* ($P=0.08$) and *Microbacterium* ($P=0.08$) were present in only bulk water samples, thus, no
321 significant microbial population differences were observed between sample types. Conversely, as
322 shown in Figure 5b, principal coordinate analysis implies that the overall microbial populations vary;
323 most of the pipe wall samples clustered together and likewise, a trend can be observed among filter

324 samples. Pinto et al. (2012) recently reported *Acidovorax*, *Hydrogenophaga* and *Denitratisoma* to be
325 associated with filter effluent and hence less impacted by disinfection processes, though our samples
326 were devoid of the latter two genera.

327

328 *Bacterial communities of sample locations*

329 The microbial community of sample locations indicated minor variability. The abundance of
330 Firmicutes ($P=0.06$) demonstrated minimal shifts based on location (Figure 3c, Supplementary Table
331 3); in the context of genera, the abundance of *Exiguobacterium* ($P=0.0009$) and *Janthinobacterium*
332 ($P=0.01$) were statistically significant among sample locations (Figure 4c). It has been reported that
333 different locations (within the same geographical region and subject to similar environmental
334 exposures) will be comprised of diverse microbial communities (Roeselers et al. 2015). As water flows
335 from one reservoir to another subjected to sequential disinfection methods it would theoretically be
336 expected that bacterial populations would shift in response. However, the shifts observed in our
337 study were not so dramatic as to significantly affect the microbial populations.

338

339 *Bacterial communities of water treatment distribution networks*

340 As mentioned, in this study there are three water distribution networks. The first receives its water
341 from Shoal Lake intake and includes the additional locations of Deacon, McPhillips, McLean and
342 Wilkes Reservoirs. The second includes the location Brandon, Manitoba and lastly, Steinbach,
343 Manitoba that utilizes water distribution systems previously described. In this regard, the abundance
344 of Proteobacteria ($P=0.05$) was highest in LIA Steinbach and lowest in HIA Brandon (Figure 3d,
345 Supplementary Table 4). *Brevundimonas* ($0.17\% \pm 0.3$; $P=0.02$), *Stenotrophomonas* ($25\% \pm 47$; $P=0.02$)
346 and *Acinetobacter* ($0.98\% \pm 1$; $P=0.03$) of Proteobacteria were highest in Steinbach (Figure 4d). The

347 abundance of Firmicutes ($P=0.05$) was significantly different among distribution systems, likely due to
348 *Staphylococcus*, which was highest in Brandon ($24\% \pm 41$; $P=0.01$). The aforementioned water
349 distribution systems receive water from different sources, consequently it is expected that the
350 microbial populations somewhat differ.

351

352 We acknowledge our study has methodological limitations; we recognize that the absence of water
353 quality characterization including microbe concentration, pH and levels of chlorine, minerals and trace
354 elements and others is a major shortcoming of this paper. Furthermore, the methods we applied to
355 enrich the samples were selected to help overcome low biomass issues. Culture enriched molecular
356 profiling has to our knowledge not been performed specifically in water but has been explored in
357 other low biomass areas, including the airway of cystic fibrosis (Sibley et al. 2011). This approach may
358 have biased the results to underrepresent some species and may partially account for the high
359 abundance of Proteobacteria.

360 **CONCLUSION**

361 Of foremost importance, we have demonstrated that microbial communities differ between incidence
362 areas of IBD within Manitoba. This is critical in attempting to elucidate the geographical variability of
363 IBD prevalence and incidence. Furthermore, we demonstrated a difference among collected sample
364 material and sample location.

365
366 The concept of drinking water potentially influencing the development and/or course of IBD has not
367 previously been recognized. Recently, the water microbiome has been well characterized (Navarro-
368 Noya et al. 2013; Lautenschlager et al. 2010; Berry et al. 2006; Roeselers et al. 2015; Lautenschlager et
369 al. 2014; Wang et al. 2014; Lührig et al. 2015; Pinto et al. 2012; Lin et al. 2014; Gomez-Alvarez et al.
370 2012), however this characterization has not extended to plausible etiologies for particular diseases
371 like IBD. Ample literature is available for waterborne acute ailments yet little to no evidence exists for
372 establishing a relationship among drinking water to chronic diseases.

373
374 The notion of a dysbiosis in the gastrointestinal tract of IBD patients is well documented and widely
375 accepted among the gastroenterological research community. What remains to be elucidated
376 however is if this dysbiosis is a cause or consequence of disease. In the case of the latter, is the
377 presence of particular microbes within the gastrointestinal tract responsible for instigating a
378 dysbiosis? And how are IBD patients acquiring these microbes? Numerous genera identified in our
379 analysis are potential human pathogens including but not limited to *Pseudomonas*, *Mycobacterium*,
380 *Stenotrophomonas*, *Aeromonas*, *Acinetobacter* and *Staphylococcus*. Therefore, we speculate drinking
381 water may contribute to IBD etiology.

382

383 It is important to highlight that the drinking water examined did not harbor potentially unsafe levels
384 of any known pathogenic bacteria and hence even differences in microbial ecology should not be
385 misconstrued as any measure of lack of safety in the drinking water of Manitoba. However, we have
386 uncovered that within the complexity of the microbial ecology of what is considered safe drinking
387 water there are differences in areas supplied by different water sources of varying incidence of IBD.
388 That having been said, we have not proved any causal associations between microbes and IBD, but
389 rather ecological associations. Our intent in this approach was not to be definitive, but instead, to
390 look for trends that could be pursued in the future. This research lays the ground work for further
391 exploration in other HIA and LIA elsewhere in Canada for similar associations as well as determination
392 as to what extent these microorganisms appear in the human gut of affected and unaffected
393 individuals.

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REFERENCES

- Aamodt, G., Bukholm, G., Jahnsen, J., Moum, B., Vatn, M.H., Lygren, I., Aubert, E., Henriksen, M., Flaaten, B., Schultz, T., Sauar, J., Kjellefold, Ø., and Stray, N. 2008. The association between water supply and inflammatory bowel disease based on a 1990-1993 cohort study in southeastern Norway. *Am. J. Epidemiol.* **168**(9):1065–1072. doi:10.1093/aje/kwn218. PMID:18801890.
- Agus, A., Massier, S., Darfeuille-michaud, A., Billard, E., and Barnich, N. 2014. Understanding host-adherent-invasive *Escherichia coli* interaction in Crohn's disease: opening up new therapeutic strategies. *Biomed. Res. Int.* **9**:e12714. doi:10.1155/2014/567929. PMID:25580435.
- Alam, M.T., Weppelmann, T.A., Weber, C.D., Johnson, J.A., Rashid, M.H., Birch, C.S., Brumback, B.A., Madsen Beau de Rochars, V.E., Glenn, J., and Ali, A. 2014. Monitoring water sources for environmental reservoirs of toxigenic *Vibrio cholerae* O1, Haiti. *Emerg. Infect. Dis.* **20**(3):356–363. doi:10.3201/eid2003.131293. PMID:24571741.
- Bai, Y., Liu, R., Liang, J., and Qu, J. 2013. Integrated metagenomic and physiochemical analyses to evaluate the potential role of microbes in the sand filter of a drinking water treatment system. *PLoS One.* **8**(4):e61011. doi:10.1371/journal.pone.0061011. PMID:23593378.
- Balzola, F., Bernstein, C., and Ho, G.T. 2011. A pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes: Commentary. *Inflamm. Bowel Dis. Monit.* **11**:166.

- Beaudeau, P., Schwartz, J., and Levin, R. 2014. Drinking water quality and hospital admissions of elderly people for gastrointestinal illness in Eastern Massachusetts, 1998-2008. *Water Res.* **52**:188–198. doi:10.1016/j.watres.2014.01.005. PMID:24486855.
- Bereschenko, L.A., Stams, A.J.M., Euverink, G.J.W., and Van Loosdrecht, M.C.M. 2010. Biofilm formation on reverse osmosis membranes is initiated and dominated by *Sphingomonas* spp. *Appl. Environ. Microbiol.* **76**(8):2623–2632. doi:10.1128/AEM.01998-09. PMID:20190090.
- Bernstein, C.N., Blanchard, J.F., Rawsthorne, P., and Wajda, A. 1999. Epidemiology of Crohn's disease and ulcerative colitis in a central Canadian province: a population-based study. *Am. J. Epidemiol.* **149**(10):916–924. PMID:10342800.
- Bernstein, C.N., Wajda, A., Svenson, L.W., MacKenzie, A., Koehoorn, M., Jackson, M., Fedorak, R., Israel, D., and Blanchard, J.F. 2006. The epidemiology of inflammatory bowel disease in Canada: A population-based study. *Am. J. Gastroenterol.* **101**(7):1559–1568. PMID:16863561.
- Berry, D., Xi, C., and Raskin, L. 2006. Microbial ecology of drinking water distribution systems. *Curr. Opin. Biotechnol.* **17**(3):297–302. PMID:16701992.
- Bhatt, A.S., Freeman, S.S., Herrera, A.F., Pedamallu, C.S., Gevers, D., Duke, F., Jung, J., Michaud, M., Walker, B.J., Young, S., Earl, A.M., Kostic, A.D., Ojesina, A.L, Hasserjian, R., Ballen, K.K., Chen, Y.B., Hobbs, G., Antin, J.H., Soiffer, R.J., Baden, L.R., Garret, W.S., Hornick, J.L., Marty, F.M., and Meyerson, M. 2013. Sequence-based discovery of *bradyrhizobium enterica* in cord colitis syndrome. *N. Engl. J. Med.* **369**(6):517–528. doi:10.1056/NEJMoa1211115. PMID:23924002.

Bitton, A., Vutcovici, M., Patenaude, V., Sewitch, M., Suissa, S., and Brassard, P. 2014. Epidemiology of inflammatory bowel disease in Québec: recent trends. *Inflamm. Bowel. Dis.* **20**(10):1770–1776. doi:10.1097/MIB.0000000000000162. PMID:25159452.

Cohn, P.D., Gleason, J.A., Rudowski, E., Tsai, S.M., Genese, C.A., and Fagliano, J.A. 2014. Community outbreak of legionellosis and an environmental investigation into a community water system. *Epidemiol. Infect.* **143**(6):1322–1331. doi:10.1017/S0950268814001964. PMID:25083716.

Dostal, A., Chassard, C., Hilty, F.M., Zimmermann, M.B., Jaeggi, T., Rossi, S., and Lacroix, C. 2012. Iron depletion and repletion with ferrous sulfate or electrolytic iron modifies the composition and metabolic activity of the gut microbiota in rats. *J. Nutr.* **142**(2):271–277. doi:10.3945/jn.111.148643. PMID:22190022.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., and Knight, R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics.* **27**(16):2194–2200. doi:10.1093/bioinformatics/btr381. PMID:21700674.

Fazli, M., Almblad, H., Rybtke, M.L., Givskov, M., Eberl, L., and Tolker-Nielsen, T. 2014. Regulation of biofilm formation in *Pseudomonas* and *Burkholderia* species. *Environ. Microbiol.* **16**(7):1961–1981. doi:10.1111/1462-2920.12448. PMID:24592823.

Frank, D.N., St Amand, A.L., Feldman, R.A., Boedeker, E.C., Harpaz, N., and Pace, N.R. 2007. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl. Acad. Sci.* **104**(34):13780–13785. PMID:17699621.

Gomez-Alvarez, V., Revetta, R.P., and Domingo, J.W.S. 2012. Metagenomic analyses of drinking water receiving different disinfection treatments. *Appl. Environ. Microbiol.* **78**(17):6095–6102. doi:10.1128/AEM.01018-12. PMID:22729545.

Green, C., Elliott, L., Beaudoin, C., and Bernstein, C.N. 2006. A population-based ecologic study of inflammatory bowel disease: Searching for etiologic clues. *Am. J. Epidemiol.* **164**(7):615–623. PMID:16920784.

Hammes, F., Berney, M., Wang, Y., Vital, M., Köster, O., and Egli, T. 2008. Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. *Water Res.* **42**(1-2):269–277. PMID:17659762.

Huang, K., Zhang, X.X, Shi, P., Wu, B., and Ren, H. 2014. A comprehensive insight into bacterial virulence in drinking water using 454 pyrosequencing and Illumina high-throughput sequencing. *Ecotoxicol. Environ. Saf.* **109**:15–21. doi:10.1016/j.ecoenv.2014.07.029. doi:10.1016/j.ecoenv.2014.07.029. PMID:25129220.

Iglesias-Rey, M., Barreiro-de Acosta, M., Caamaño-Isorna, F., Rodríguez, I.V., Ferreiro, R., Lindkvist, B., González, A.L., and Dominguez-Munoz, J.E. 2014. Psychological factors are associated with changes in the health-related quality of life in inflammatory bowel disease. *Inflamm. Bowel. Dis.* **20**(1):92–102. doi:10.1097/01.MIB.0000436955.78220.bc. PMID:24193152.

Kotlowski, R., Bernstein, C.N., Sepehri, S., and Krause, D.O. 2007. High prevalence of *Escherichia coli* belonging to the B2+D phylogenetic group in inflammatory bowel disease. *Gut.* **56**(5):669–675. PMID:17028128.

Lautenschlager, K., Boon, N., Wang, Y., Egli, T., and Hammes, F. 2010. Overnight stagnation of drinking water in household taps induces microbial growth and changes in community composition. *Water Res.* **44**(17):4868–4877. doi:10.1016/j.watres.2010.07.032. PMID:20696451.

Lautenschlager, K., Hwang, C., Ling, F., Liu, W.T., Boon, N., Köster, O., Egli, T., and Hammes, F. 2014. Abundance and composition of indigenous bacterial communities in a multi-step biofiltration-based drinking water treatment plant. *Water Res.* **62**:40–52. doi:10.1016/j.watres.2014.05.035. PMID:24937356.

Lee, C.S., Wetzel, K., Buckley, T., Wozniak, D., and Lee, J. 2011. Rapid and sensitive detection of *Pseudomonas aeruginosa* in chlorinated water and aerosols targeting *gyrB* gene using real-time PCR. *J. Appl. Microbiol.* **111**(4):893–903. doi:10.1111/j.1365-2672.2011.05107.x. PMID:21794031.

Lin, W., Yu, Z., Zhang, H., and Thompson, I.P. 2014. Diversity and dynamics of microbial communities at each step of treatment plant for potable water generation. *Water Res.* **52**:218–230. doi:10.1016/j.watres.2013.10.071. PMID:24268295.

Liu, G., Bakker, G., Li, S., Vreeburg, J., Verberk, J.Q.J.C., Medema, G., Liu, W.T., and Van Dijk, J.C. 2014. Integral study of microbial ecology of unchlorinated drinking water distribution system: analysis of structure and composition of bacterial communities in bulk water, suspended solids, loose deposits and pipe wall biofilm. *Environ. Sci. Technol.* **48**:5467–5476.

Lührig, K., Canbäck, B., Paul, C.J., Johansson, T., Persson, K.M., and Rådström, P. 2015. Bacterial community analysis of drinking water biofilms in southern Sweden. *Microbes Environ.* **30**(1):99–107. doi:10.1264/jsme2.ME14123. PMID:25739379.

- McMurdie, P.J., and Holmes, S. 2013. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*. **8**(4):e61217. doi:10.1371/journal.pone.0061217. PMID:23630581.
- Molodecky, N.A., Soon, I.S., Rabi, D.M., Ghali, W.A., Ferris, M., Chernoff, G., Benchimol, E.I., Panaccione, R., Ghosh, S., Barkema, H.W., and Kaplan, G.G. 2012. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology*. **142**(1):46–54.e42. doi:10.1053/j.gastro.2011.10.001. PMID:22001864.
- Navarro-Noya, Y.E., Suárez-Arriaga, M.C., Rojas-Valdes, A., Montoya-Ciriaco, N.M., Gómez-Acata, S., Fernández-Luqueño, F., and Dendooven, L. 2013. Pyrosequencing analysis of the bacterial community in drinking water wells. *Microb. Ecol.* **66**(1):19–29. doi:10.1007/s00248-013-0222-3. PMID:23563631.
- Nazareth, N., Magro, F., Appelberg, R., Silva, J., Gracio, D., Coelho, R., Cabral, J.M., Abreu, C., Macedo, G., Bull, T.J., and Sarmiento, A. 2015. Increased viability but decreased culturability of *Mycobacterium avium* subsp. *paratuberculosis* in macrophages from inflammatory bowel disease patients under Infliximab treatment. *Med. Microbiol. Immunol.* **204**(6):647–656. doi: 10.1007/s00430-015-0393-2. PMID:25702170.
- Newton, R.J., Jones, S.E., Eiler, A., McMahon, K.D., and Bertilsson, S. 2011. A Guide to the Natural History of Freshwater Lake Bacteria. *Microbiol. Mol. Biol. Rev.* **75**(1):14-49. doi:10.1128/MMBR.00028-10. PMID:21372319.
- Osterman, M.T., Sandborn, W.J., Colombel, J.F., Robinson, A.M., Lau, W., Huang, B., Pollack, P.F., Thakkar, R.B., and Lewis, J.D. 2014. Increased risk of malignancy with adalimumab combination therapy, compared with monotherapy, for Crohn's disease. *Gastroenterology*. **146**(4):941–949.e2. doi:10.1053/j.gastro.2013.12.025.
- Parkes, G.C., Whelan, K., and Lindsay, J.O. 2014. Smoking in inflammatory bowel disease: Impact on disease course and insights into the aetiology of its effect. *J. Crohn's Colitis*. **8**:717–725. PMID:24361468.

Pinto, A.J., Xi, C., and Raskin, L. 2012. Bacterial community structure in the drinking water microbiome is governed by filtration processes. *Environ. Sci. Technol.* **46**(16):8851–8859. doi:10.1021/es302042t. PMID:22793041.

Prideaux, L., De Cruz P., Ng, S.C., and Kamm, M.A. 2012. Serological antibodies in inflammatory bowel disease. *Inflamm. Bowel. Dis.* 2012, **18**(7):1340–1355. doi:10.1002/ibd.21903. PMID:22069240.

Pruesse, E., Quast, C., Knittel, K., Fuchs, B.M., Ludwig, W., Peplies, J., and Glöckner, F.O. 2007. SILVA: A comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res.* **35**(21):7188–7196. PMID:17947321.

Rhodes, G., Henrys, P., Thomson, B.C., and Pickup, R.W. 2013. *Mycobacterium avium* subspecies paratuberculosis is widely distributed in British soils and waters: Implications for animal and human health. *Environ. Microbiol.* **15**(10):2761–2774. doi:10.1111/1462-2920.12137. PMID:23648004.

Roeselers, G., Coolen, J., van der Wielen, P.W.J.J., Jaspers, M.C., Atsma, A., de Graaf, B., and Schuren, F. 2015. Microbial biogeography of drinking water: patterns in phylogenetic diversity across space and time. *Environ. Microbiol.* **17**(7):2505–2514. doi:10.1111/1462-2920.12739. PMID:25581482.

Rudi, K., Berg, F., Gaustad, E., Tannes, T., and Vatn, M. 2010. Ratios between Alpha-, Beta- and Gamma-proteobacteria in tap water determined by the ProteoQuant assay. *Lett. Appl. Microbiol.* **50**(1):1–6. doi:10.1111/j.1472-765X.2009.02743.x. PMID:19874479.

Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F., Turner, P., Parkhill, J., Loman, N.J., and Walker, A.W. 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biol.* **12**;12:87. doi:10.1186/s12915-014-0087-z. PMID:25387460.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., and Weber, C.F. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **75**(23):7537–7541. doi:10.1128/AEM.01541-09. PMID:19801464.

Sibley, C.D., Grinwis, M.E., Field, T.R., Eshaghurshan, C.S., Faria, M.M., Dowd, S.E., Parkins, M.D., Rabin, H.R., and Surette, M.G. 2011. Culture enriched molecular profiling of the cystic fibrosis airway microbiome. *PLoS One.* **6**(7):25–27. doi: 10.1371/journal.pone.0022702. PMID:21829484.

Simões, L.C., Simões, M., and Vieira, M.J. 2010. Influence of the diversity of bacterial isolates from drinking water on resistance of biofilms to disinfection. *Appl. Environ. Microbiol.* **76**(19):6673–6679. doi:10.1128/AEM.00872-10. PMID:20693444.

Suwandi, A., Bargaen, I., Roy, B., Pils, M.C., Krey, M., Zur Lage, S., Basler, T., Rohde, M., Falk, C.S., Hornef, M.W., Goethe, R., and Weiss, S. 2014. Experimental colitis is exacerbated by concomitant infection with *Mycobacterium avium* ssp. *paratuberculosis*. *Inflamm. Bowel. Dis.* **20**(11):1962–1971. doi:10.1097/MIB.0000000000000157. PMID:25144571.

Thomas, J.M., and Ashbolt, N.J. 2011. Do free-living amoebae in treated drinking water systems present an emerging health risk? *Environ. Sci. Technol.* **45**(3):860–869. doi:10.1021/es102876y. PMID:21194220.

- Vagianos, K., Clara, I., Carr, R., Graff, L.A., Walker, J.R., Targownik, L.E., Lix, L.M., Rogala, L., Miller, N., and Bernstein, C.N. 2016. What are adults with inflammatory bowel disease (IBD) eating? A closer look at the dietary habits of a population-based Canadian IBD cohort. *J. Parenter. Enter. Nutr.* **40**(3):405-11. doi:10.1177/0148607114549254. PMID:25189173.
- Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. 2007. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **73**(16):5261–5267. PMID:17586664.
- Wang, H., Masters, S., Edwards, M.A., Falkinham, J.O., and Pruden, A. 2014. Effect of disinfectant, water age, and pipe materials on bacterial and eukaryotic community structure in drinking water biofilm. *Environ. Sci. Technol.* **48**(3):1426–1435. doi:10.1021/es402636u. PMID:24401122.
- Wei, B., Huang, T., Dalwadi, H., Sutton, C.L., Bruckner, D., and Braun, J. 2002. *Pseudomonas fluorescens* encodes the Crohn's disease-associated I2 sequence and T-cell superantigen. *Infect. Immun.* **70**(12):6567–6575. PMID:12438326.
- Wingender, J., and Flemming, H.C. 2011. Biofilms in drinking water and their role as reservoir for pathogens. *Int. J. Hyg. Environ. Health.* **214**(6):417–423. doi:10.1016/j.ijheh.2011.05.009. PMID:21697011.

Figure 1. Map of Manitoba, Canada illustrating incidence of IBD. Incidence (number of cases per 100,000 population) of Crohn's disease (top) and ulcerative colitis (bottom) in the province of Manitoba and the City of Winnipeg, Manitoba, Canada, 1990–2001. Results were age-standardized to the 1996 Manitoba population. Adapted with permission from Green et al. (2006).

Figure 2. Plot of alpha-diversity metrics. Plots demonstrate community richness (estimated by ACE and Chao1) and diversity (estimated by Shannon, Simpson and inverse-Simpson) variation observed between A) low and high incidence areas B) sample types C) sample locations and D) water distribution networks. Differences considered significant at $*=P<0.05$.

Figure 3. Phylum-level abundances of the water microbiota. Comparison of phyla abundance as documented between A) low and high incidence areas B) sample types C) sample locations and D) water distribution networks. Phyla are expressed as average abundance. Differences considered significant at $*=P<0.05$.

Figure 4. Genus-level abundances of the water microbiota. Comparison of genera abundance as documented between A) low and high incidence areas B) sample types C) sample locations and D) water distribution networks. Genera are expressed as average abundance. Differences considered significant at $*=P<0.05$

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85x149mm (150 x 150 DPI)

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1763x917mm (72 x 72 DPI)

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