Antibiotic resistome and microbial community structure during anaerobic co-digestion of food waste, paper and cardboard

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16 ABSTRACT

17 Antimicrobial resistance is a globally recognized public health risk. High incidence of
18 antibiotic resistant bacteria and antibiotic resistance genes (ARGs) in solid organic waste
19 necessitates the development of effective treatment strategies. The objective of this study
20 was to assess ARG diversity and abundance as well as the relationship between resistome
21 and microbial community structure during anaerobic co-digestion (AD) of food waste,
22 paper and cardboard. A lab-scale solid-state AD system consisting of six sequentially fed
23 leach beds (each with a solids retention time of 42 days) and an upflow anaerobic sludge
24 blanket (UASB) reactor was operated under mesophilic conditions continuously for 88
25 weeks to successfully treat municipal organic waste and produce biogas. A total of ten
26 samples from digester feed and digestion products were collected for microbial
27 community analysis including SSU rRNA gene sequencing, total community
28 metagenome sequencing and quantitative PCR. Taxonomic analyses revealed that AD
29 changed the taxonomic profile of the microbial community: digester feed was dominated
30 by bacterial and eukaryotic taxa while anaerobic digestate possessed a large proportion of
31 archaea mainly belonging to the methanogenic genus *Methanosaeta.* ARGs were
32 identified in all samples with significantly higher richness and relative abundance per 16S
33 rRNA gene in digester feed compared to digestion products. Multidrug resistance was the
34 most abundant ARG type. AD was not able to completely remove ARGs as shown by
35 ARGs detected in digestion products. Using metagenomic assembly and binning we
36 detected potential bacterial hosts of ARGs in digester feed, that included *Erwinia*,
37 *Bifidobacteriaceae*, *Lactococcus lactis* and *Lactobacillus.*

38

IMPORTANCE

Solid organic waste is a significant source of antibiotic resistance genes (ARGs) (1) and effective treatment strategies are urgently required to limit the spread of antimicrobial resistance. Here we studied the antibiotic resistome and microbial community structure within an anaerobic digester treating a mixture of food waste, paper and cardboard. We observed a significant shift in microbial community composition and a reduction in ARG diversity and abundance after 6 weeks of digestion. We identified the host organisms of some of the ARGs including potentially pathogenic as well as non-pathogenic bacteria, and we detected mobile genetic elements required for horizontal gene transfer. Our results indicate that the process of sequential solid-state anaerobic digestion of food waste, paper and cardboard tested herein provides a significant reduction in the relative abundance of ARGs per 16S rRNA gene.

INTRODUCTION

Antimicrobial resistance (AMR) is a widely recognized public health risk. The extensive use of antimicrobial compounds since World War II has triggered the rapid spread and evolution of AMR mechanisms to such an extent that AMR is considered one of today's top medical concerns globally (2). Currently, 700 000 deaths are attributed to resistant microbial infections annually, which is projected to increase to 10 million deaths by 2050 resulting in the loss of 100 trillion USD of economic output (3). In light of this gloomy prospect, a One-Health approach has been proposed to tackle AMR by recognizing the connections between human and animal health and the environment (4).

Although the vast majority of antimicrobial research is focusing on the clinical setting, the natural environment has gained attention as a possible reservoir and dispersal route of antimicrobial resistance determinants, including antibiotic resistant bacteria and antibiotic resistance genes (ARGs) (5, 6). Various natural ecosystems, such as ancient permafrost sediments (7), pristine soil (8, 9), oceanic (10) and freshwater bodies (11) have been shown to possess a diversity of ARGs collectively defined as the resistome. However, higher concentrations of resistance determinants have been observed in environments with anthropogenic impact where selective pressure for ARGs is present, such as wastewater treatment plants, animal husbandry facilities, aquaculture farms and pharmaceutical manufacturing (12).

Various types of solid organic waste may also serve as potential sources of antibiotic resistant bacteria and ARGs (1). In addition to the extensively studied resistomes of sewage sludge and animal manure, the organic fraction of municipal solid waste (MSW) may also contribute to the dissemination of AMR. Global production of

MSW is estimated at around 2 billion tons per year, of which 34–53% is organic biodegradable waste including food waste as the primary component (13). Paper waste forms 17% of MSW globally, including different lignocellulosic fibres such as cardboard, newspaper, magazines, wrapping paper, shredded paper, boxes, bags and beverage cups (14).

Several studies have highlighted the role of the food chain in AMR as a direct link to human health (15, 16) and identified antibiotic resistant pathogens in various food products such as meat (17–19), fruit and vegetables (20–23), poultry (24, 25), fish (26, 27) and dairy (28). Therefore, it is reasonable to assume that ARGs are also present in food waste: Lee *et al*. (29) detected a variety of ARGs in the wastewater from food waste recycling in Korea, although the abundance of ARGs remained below that of manure and sewage sludge.

To mitigate the harmful environmental impacts of organic solid waste, anaerobic digestion (AD) is widely implemented as a treatment strategy, providing waste stabilization as well as the production of renewable energy (30). AD changes the structure of the microbial community of the substrates which in turn affects the resistome present in digestion products. Although it is generally accepted that ARG abundance is reduced overall during AD, enrichment of some ARGs and rebound-effects have been reported in several studies (summarized by Youngquist *et al.* (31)). For example, Pu *et al.* (32) studied the impact of applying pig manure to fields and found that AD reduced the relative abundance of Macrolide-Lincosamide-Streptogramin (MLS) and tetracycline resistance genes, while resistance genes for sulfa, aminoglycoside, florfenicol, amphenicol and chloramphenicol were enriched. Similarly, the effect of aerobic

98 composting of organic waste remains contradictory: while some evidence suggests
99 composting can reduce the abundance of antibiotic resistant bacteria and ARGs, other
100 studies have shown increased abundance and diversity of ARGs (31).

101 Recently, there has been growing interest in AD of food waste due to its high
102 energetic value (13). However, limited information is available on the effect of AD on the
103 microbial community and resistome in food waste. Zhang *et al*. (33) identified 11 ARGs
104 and class 1 integron integrase gene *intI1* in digested food waste: while *tetA*, *tetB*, *tetX*,
105 *sul1*, *cmlA*, *floR* and *intI1* were significantly reduced by AD, enrichment of *tetM*, *tetW*,
106 *tetQ* and *tetO* was recorded. Similarly, another study detected both increase and reduction
107 of specific ARGs in the co-digestion of sewage sludge and food waste following
108 microwave pretreatment (34). Thus, the effect of AD on the food waste resistome and the
109 associated microbial community is still unclear.

110 In addition to quantification of individual ARGs, detecting potential host
111 organisms of these genes is important to evaluate the risk to human health. Several
112 studies have used correlation and network analysis to detect relationships between
113 individual ARGs and bacterial genera (34–37). High mobility of ARGs due to horizontal
114 gene transfer (HGT) is responsible for the spread of AMR between different bacteria
115 including human pathogens as well as non-pathogenic environmental bacteria which
116 often serve as reservoirs of ARGs (38).

117 The objective of this study was to measure ARG abundance and resistome
118 diversity before and after anaerobic co-digestion of food waste, paper and cardboard.
119 Samples were collected from a lab-scale solid-state AD system that exhibited stable
120 methane production and substrate destruction rates (39, 40). By simultaneously analyzing

121 microbial community shifts, we attempted to assign ARGs to specific host organisms.
122 Using SSU rRNA gene sequencing, total community metagenome sequencing and
123 targeted quantitative PCR we were able to quantify changes in the diversity and size of
124 the resistome before and after digestion together with changes in the taxonomic profile of
125 the microbial community. We were further able to identify potential host organisms of
126 ARGs by using metagenomic assembly and binning methods.
127
128 RESULTS
129 A total of 10 samples were collected from a lab-scale solid-state anaerobic digestion
130 system described in detail by a recent PhD thesis (39). The ten samples included three
131 samples of raw food waste (FW1, FW2, FW3) collected from local residential green bin
132 program, three samples of the mixture of food waste and lignocellulosic fibres that
133 constituted the leach bed feed (LBF1, LBF2, LBF3), three samples of 6 week old
134 digestate (DG74, DG76, DG78) and one sample of the microbial granules from the
135 upflow anaerobic sludge blanket reactor (UASB) treating leachate from the leach beds.
136 DNA was extracted from each sample in order to analyze the microbial community
137 composition and ARGs diversity, abundance, genomic location and host organisms using
138 a multi-pronged approach combining metagenome sequence analyses, taxonomic
139 profiling and quantitative PCR (Fig. 1).
140 **Microbial community.** Microbial community structure was analyzed by small
141 subunit (SSU) rRNA gene-fragment sequencing (method 2 in Fig. 1) capturing bacterial,
142 archaeal and eukaryotic diversity (Tables S1 and S2 in supplemental material). A clear
143 distinction in the communities of digester feed and digestion products was detected: food

144 waste and leach bed feed microbial communities were dominated by bacterial and
145 eukaryotic taxa while anaerobic digestate and microbial community of granules from the
146 UASB reactor possessed a large proportion of archaeal phylotypes mainly belonging to
147 the methanogenic genus *Methanosaeta* (Fig. 2). The most abundant bacterial OTUs in
148 digester feed belonged to the family *Enterobacteriaceae*, including the genera
149 *Citrobacter*, *Enterobacter*, *Erwinia*, *Kluyvera*, *Pantoea*, *Serratia* and *Lelliottia*.
150 Additionally, representatives of the phylum *Firmicutes* were detected in FW2, including
151 the genera *Lactobacillus* and *Leuconostoc*, probably indicating fermentation processes
152 occurring in the respective food waste. In addition to bacterial sequences, fungal and
153 plant material was detected in digester feed (FW, LBF), but not in digestion products
154 (DG) or the UASB. Across all samples, 36–62% of OTUs were detected at low relative
155 abundances comprising less than 1% of the total community. A comparison of genus
156 level taxonomic composition detected from SSU rRNA gene amplicon sequence analysis
157 using QIIME (tool 2.1 in Fig. 1) to that derived from total community metagenome
158 sequencing analysis using Kaiju (tool 3.3 in Fig. 1) or metaxa2 (tool 3.4 in Fig. 1)
159 showed relatively good agreement between the three different annotation tools used in
160 this study (Fig. S1 in supplemental material).

161 **ARG diversity and abundance.** Total community metagenome sequencing data
162 from seven samples (Table S3 in supplemental material) was analyzed using the ARGs
163 Online Analysis Pipeline (ARGs-OAP) (41) (tool 3.5 in Fig. 1) to characterize the
164 distribution and diversity of ARGs before and after anaerobic digestion. The richness of
165 ARGs, measured as the number of distinct ARGs identified in one sample type, was
166 highest in digester feed with 330 and 336 different ARGs detected in FW and LBF

167 samples, respectively. The richness of ARGs in digestion products remained two times
168 lower with 115 different ARGs detected in DG and UASB samples indicating reduced
169 diversity of ARGs after anaerobic digestion. Twenty three ARGs were found to be unique
170 to digestion products belonging to aminoglycoside (*aac(3)-I*, *ant(9)-I*), beta-lactam
171 (*OXA-10*, *OXA-205*, *OXA-251*, *OXA-34*, *OXA-46*, *OXA-75*), chloramphenicol (*catQ*),
172 MLS (*ereB*, *lnuB*, *mphA*, *carA*), sulfonamide (*sul3*), tetracycline (*tet44*, *tetT*),
173 trimethoprim (*dfrA5*) and vancomycin (*vanA*, *vanG*, *vanH*, *vanN*, *vanU*, *vanX*) type.
174 In addition to richness, the relative abundance of ARGs per 16S rRNA gene was
175 determined from total community metagenome sequencing data (Table S4 in
176 supplemental material). Relative abundances of ARGs per 16S rRNA gene were
177 significantly higher in digester feed than in digestion products, indicating the ability of
178 anaerobic digestion to reduce ARGs (Fig. 3). Total relative abundances of ARGs per 16S
179 rRNA gene in food waste samples FW1 (0.78 ARG/16S rRNA) and FW2 (0.40 ARG/16S
180 rRNA) were similar to the respective values in the mixtures of food waste with
181 lignocellulosic fibres (LBF1 0.84 ARG/16S rRNA, LBF2 0.44 ARG/16S rRNA),
182 indicating the role of food waste as the primary source of ARGs in leach bed feed. Most
183 of the ARGs identified in digester feed conferred multidrug resistance (Fig. 3A).
184 Additionally, many potential ARGs with unclassified resistance type were detected. The
185 highest relative abundance value for an individual gene (Fig. 3B) was recorded for
186 AcrAB-TolC multidrug efflux complex subunit *acrB* reaching 0.05 copies/16S rRNA
187 gene in samples FW1 and LBF1. Other highly abundant genes in digester feed included
188 subunits of multidrug resistance complexes (*acrA*, *mdtB*, *mdtC*, *tolC*), several regulatory
189 protein genes associated with AMR (*cpxR*, *arlR*) and bacitracin resistance gene *bacA*.

190 Relative abundances of individual ARGs in digestion products remained below 0.01
191 copies/16S rRNA in all cases. Similarly to digester feed, the highest value for an
192 individual gene in digestion products was recorded for *acrB* with 0.007 copies/16S rRNA
193 in UASB.

194 A high-throughput qPCR (HT-qPCR) array was also used as an alternative
195 approach to quantify 315 ARGs (tool 4.1 in Fig. 1) and 57 mobile genetic elements
196 (MGEs) (tool 4.2 in Fig.1) in food waste and digestate DNA samples (Table S5 in
197 supplemental material). In agreement with the results from the metagenomic data, the
198 diversity of ARGs and MGEs was higher in food waste with 161 different genes detected,
199 while only 32 different genes were detected in digestate (Fig. 4). Among these, 10 ARGs
200 were found only in digestate samples and not in food waste. Notably, MLS resistance
201 genes *mphA* and *lnuB*, phenicol resistance gene *catQ* and tetracycline resistance gene
202 *tet44* were found to be unique to digestate by both methods.

203 Although the richness of ARGs and MGEs was higher in food waste samples,
204 absolute abundances of individual genes normalized per sample dry weight reached
205 higher levels in digestates (Fig. 4) according to HT-qPCR array results calculated based
206 on abundance of 16S rRNA gene per gram of dry sample (tool 1.1 in Fig. 1, Fig. S2 in
207 supplemental material). Most prevalent ARG types in final digestates (Fig. 4B) included
208 MLS (genes *ere(A)*, *erm(F)*, *erm(O)*, *lnu(F)*, *lnuB*, *mef(B)*, *mphA*), aminoglycoside
209 (genes *aac3-Via*, *aadE*, *aph4ib*) and tetracycline (genes *tet44*, *tetD*, *tetM*, *tetW*) with
210 absolute abundances of individual genes ranging between 10^7 and 10^8 copies/g-dw.
211 Absolute abundances of ARGs in food waste samples ranged between 10^4 and 10^7
212 copies/g-dw. MGEs followed a similar pattern to ARGs with more genes detected in food

213 waste but higher absolute abundances of individual genes per gram of sample dry weight
214 detected in digestates (Fig. 4C).

215 **Genomic context of ARGs.** In order to study the genomic context of ARGs, total
216 community metagenome sequencing reads were assembled into longer contigs using
217 MEGAHIT (tool 3.6 in Fig. 1) and metaSPAdes (tool 3.7 in Fig. 1) assemblers (Table S6
218 in supplemental material), followed by detection of ARGs on contigs (tool 3.10 in Fig. 1)
219 and identification of plasmid sequences carrying ARGs (tool 3.8 in Fig. 1) (Table S7 in
220 supplemental material).

221 On average, 0.10% of all assembled contigs carried ARGs in digester feed, while
222 only 0.003% of contigs identified in digestion products included ARGs (Table S8 in
223 supplemental material). More than 90% of ARG-carrying contigs in all samples included
224 only one ARG, however, contigs with multiple ARGs were also observed with up to five
225 ARGs per contig (Table S8 in supplemental material) detected in digester feed. Contigs
226 with multiple ARGs typically carried subunits of multidrug efflux systems such as
227 MdtABC-TolC coupled with two-component regulatory systems for efflux proteins (such
228 as BaeSR).

229 Identification of plasmids from ARG-carrying contigs revealed that on average
230 32% of ARGs detected in digestion substrates were located on plasmids (Table 1)
231 indicating their potential for HGT. The distribution of plasmid ARGs resembled the
232 pattern of ARGs identified from short sequencing reads with multidrug resistance genes
233 being the most abundant type in digestion substrates (Fig. S3 in supplemental material).
234 Proportion of plasmid ARGs in digestion products varied between 33.3% and 60.0%,
235 although the number of detected ARGs remained low (11 ARGs on plasmids in DG74,

24 ARGs in DG78, and 20 ARGs in UASB, respectively; Table 1). ARGs identified as chromosomal accounted for 8.3 to 28.9% of ARGs across all samples, remaining below the respective values for plasmid ARGs in all cases (Table 1).

Bacterial hosts of ARGs. MEGAHIT-assembled contigs that were not identified as plasmids were further annotated for their taxonomic affiliation (tool 3.9 in Fig. 1). Figure 5 depicts the frequency of specific host-ARG pairs identified for most abundant bacterial genera in digester feed. Gammaproteobacterial genera *Stenotrophomonas* and *Acinetobacter* clustered separately and were characterized by species-specific multidrug resistance efflux complexes and the corresponding regulatory systems. *Stenotrophomonas* carried subunits for SmeABC and SmeDEF efflux pumps as well as the SmeRS regulatory system formerly identified in *S. maltophilia* (42, 43). *Acinetobacter* carried subunits for AdeIJK and AdeFGH multidrug efflux pumps together with multidrug resistance genes *adeB*, *abeM*, transcriptional activator *mexT* and aminoglycoside resistance gene *APH(3')-Ia*. In addition to *Stenotrophomonas* and *Acinetobacter*, other identified ARG host genera in the digester feed included *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Raoultella*, *Rahnella*, *Rouxiella*, *Pantoea*, *Serratia*, *Erwinia*, *Citrobacter*, *Leclercia* and *Lelliottia* that formed a network by sharing connections with ARGs belonging mainly to multidrug resistance type. For example, *Pseudomonas* was characterized by the highest number of ARG connections carrying subunits of the MexEF-OprN efflux system; similarly, *Enterobacter* and *Klebsiella* shared connections to subunits of MdtABC-TolC and AcrAB-TolC efflux systems. In addition to multidrug resistance genes, genes conferring resistance to polymyxin were related to multiple host genera in digester feed: *rosA* and *rosB* were both found in

Serratia, *Rahnella* and *Rouxiella*, *arnA* was found in *Citrobacter* and *Pseudomonas*, *pmrE* in *Enterobacter* and *Klebsiella*, and *pmrA* in *Raoultella*.

Only a limited number of ARGs were detected and their host organisms taxonomically determined in digestion products (Table 2). Bacterial hosts of ARGs in digestion products included genera formerly identified in anaerobic digesters associated with microbial degradation processes, such as *Fermentimonas*, and several sulphate-reducing genera (*Desulfobacca*, *Desulfococcus*). More importantly, *Burkholderia* and *Arcobacter* (both carrying isoniazid resistance gene *katG*), *Streptococcus* (carrying MLS resistance gene *mefA*) and *Escherichia* (carrying MLS resistance gene *mefB*) were identified as ARG host organisms of clinical relevance.

To further investigate the potential hosts of ARGs, the assembled contigs were binned into 201 metagenome assembled genomes (MAGs) (tools 3.11, 3.12 and 3.13 in Fig. 1) with >75% completeness and <25% redundancy (Table S9, Fig. S4 in supplemental material). Notably, MAGs found in digestion products (DG74, DG78, UASB) accounted for 75% of all assembled MAGs and did not contain ARGs. On the contrary, four MAGs containing ARGs were identified in digester feed (FW1, FW2, LBF1, LBF2) (Table 3). Interestingly, a genome belonging to the plant pathogenic genus *Erwinia* carried 19 ARGs conferring resistance to aminoglycoside, bacitracin, polymyxin, quinolone and sulfonamide antibiotics while also harboring several genes for multidrug resistance. Lactic acid bacteria *Lactococcus lactis* and *Lactobacillus* that are generally associated with probiotic features displayed resistance for MLS (*Lactococcus lactis* genes *lmrC*, *lmrD*, *lmrP*), tetracycline (*Lactococcus lactis* genes *tetM*, *tetS*) and trimethoprim (*Lactobacillus* gene *dfrE*). Additionally, a MAG belonging to the family

282 *Bifidobacteriaceae* carried the inner membrane transporter gene *mdsB* of multidrug and
283 metal efflux complex MdsABC. The four ARG-containing MAGs that were highly
284 abundant in digester feed were not identified in digestion products (Fig. S4 in
285 supplemental material).
286
287 DISCUSSION
288 AD is widely used for treatment of solid organic waste providing waste stabilization as
289 well as energy (biogas) production. More recently, the potential of AD to reduce
290 antibiotic resistant bacteria and ARGs has been investigated with mixed results (31). In
291 this study, we examined the effect of co-digestion of food waste, paper and cardboard on
292 microbial community composition and resistome using SSU rRNA gene sequencing, total
293 community metagenome sequencing and quantitative PCR. The studied AD system that
294 was a lab-scale solid-state leach bed reactor with leachate recycle via a UASB reactor
295 that has been thoroughly described elsewhere (40). This AD system performed very well
296 over 1.5 years of operation, also maintaining a stable microbial community (44).
297 **Microbial community composition of digester feed and digestion products.**
298 Microbial community composition has been suggested as one of the main drivers of
299 ARGs in anaerobic digesters (35, 45). The microbial communities of the digester feed – a
300 mix of food waste and lignocellulosic fibres – revealed aerobes and facultative aerobes
301 that were distinct from the strictly anaerobic bacteria and archaea inside the digester. The
302 FW alone and the blended LBF samples had very similar taxonomic profiles indicating
303 that food waste was the main contributor to the microbial community in the digester feed,

304 consisting mainly of microorganisms native to food products or of the microbes that
305 colonized food waste during collection and storage prior to AD.

306 Previous studies on food microbiomes have highlighted the role of fermentative
307 organisms such as lactic acid bacteria in fermentation processes (46). Several OTUs
308 belonging to the genera *Lactobacillus* and *Leuconostoc* were identified in the food waste
309 used in this study, indicating the presence of fermented food products (e.g. cheese,
310 sourdough) or the start of degradation processes during the collection, storage and pre-
311 processing of food waste prior to AD. *Lactobacillus* and *Leuconostoc* may also indicate
312 spoilage of meat as previously shown for vacuum-packed pork (47), beef (48–50),
313 minced meat (51) and sausages (52, 53). Besides lactic acid bacteria, several genera from
314 the family *Enterobacteriaceae* were detected in digester feed, including *Citrobacter*,
315 *Enterobacter*, *Erwinia*, *Kluyvera*, *Pantoea*, *Serratia* and *Lelliottia*. Jackson *et al*. (54)
316 identified *Serratia*, *Erwinia*, *Enterobacter* and *Pantoea* from leafy salad vegetables,
317 which are also common in food waste. While many of the identified *Enterobacteriaceae*
318 are widely recognized as plant pathogens or symbionts (e.g. *Erwinia*), potential human
319 pathogens were also detected (e.g. *Enterobacter*, *Serratia*).

320 Special attention should be given to the occurrence of ESKAPE pathogens -
321 *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter*
322 *baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species, that are common causes
323 of nosocomial infections and characterized by various antimicrobial resistance
324 mechanisms (55). A total of 15 OTUs annotated to *S. aureus*, *K. pneumoniae*, *A.*
325 *baumannii* or *P. aeruginosa* were found in very low abundance in digester feed (each
326 with relative abundance below 0.12% of total OTUs), while none of these were detected

in digestion products. One hundred and two (102) OTUs were annotated to *Enterobacter* comprising up to 0.13% of OTUs in digester feed, while none was detected in digestion products. Thus, ESKAPE pathogens were detectable in relatively low abundance in the digester feed used in this study but did not survive anaerobic treatment.

The microbial community after AD was completely different from that in the feed. Hydrolytic, fermenting, acidogenic, acetogenic and methanogenic microorganisms comprise a typical microbial community in a stable AD process. Representatives from the phyla *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes* and *Proteobacteria* that have been connected to hydrolysis, fermentation and acidogenesis (56) were identified in the bacterial communities of the digestate samples in this study. As expected, digestates exhibited abundant archaeal communities responsible for methane production. Acetoclastic methanogen *Methanosaeta* was identified as the dominant archaeal genus (reaching up to 72.6% of total archaeal OTUs in DG74), which has been noted before for digesters with high acetate concentration (57, 58). Members of the orders *Methanobacteriales* and *Methanomicrobiales* commonly identified in anaerobic digesters treating food waste (59–61) were also present in the digestates of the current study. Thus, a diverse methanogenic community achieved stable methane production, as evidenced by substantial feedstock degradation and biogas production rates (40).

ARGs in digester feed and digestion products. ARGs were identified in all samples, in digester feed as well as in AD products. Higher richness as well as higher relative abundance of individual ARGs per 16S rRNA gene was found in digester feed compared to digestion products indicating the ability of AD to reduce ARG diversity and abundance. Total relative abundance of ARGs per 16S rRNA gene reached 0.84

ARG/16S rRNA gene in digester feed (LBF1), which is comparable to ARG levels in faeces and wastewater from livestock farms (36), while the respective value in digestion products was below 0.05 ARG/16S rRNA (0.04 ARG/16S rRNA in UASB), comparable to natural environments such as soils, sediments and river water (36). Thus, AD clearly reduced ARG values from levels associated with high contamination to environmental background levels.

Yet, although AD significantly reduced the abundance of ARGs, a limited number of ARGs were also detected in digestion products. Total community metagenome sequencing and HT-qPCR array both detected ARGs in digestion products that were low in abundance relative to the total microbial population (as shown by total community metagenome sequencing), but achieved high absolute abundance per gram dry mass of digestate (reaching 10^8 copies/g-dw for individual genes and 10^9 copies/g-dw in total) due to high bacterial loads in the digestates (as shown by HT-qPCR array). Same order of magnitude absolute abundance values of individual ARGs detected by qPCR have also been reported previously for anaerobic digestates of FW (62). Thus, the effectiveness of AD on treating waste containing ARGs is a function of which microbes can grow in the AD system. In this case study, ARGs from food waste microbes were eliminated while ARGs present in the microbes involved in the digestion process were more abundant in the final digestate, and were at high absolute abundance owing to the high concentration of microbes generally after digestion. Future experiments should focus on post-treatment of digested solids, such as additional aerobic curing to reduce odor and stabilize waste, to determine the ultimate environmental load of ARGs associated with land application of anaerobic digestates.

373 Special attention should be given to the mobility of ARGs that can be transferred
374 from environmental bacteria to human pathogens or vice versa as shown previously (63).
375 In this study, on average, 32% of ARGs in assembled contigs from digester feed were
376 located on plasmids, indicating their potential mobility. Similar plasmid proportions from
377 total assembled contigs have also been found in photobioreactor microbial communities
378 (64) and microbial mats inhabiting mine waters (65). The HT-qPCR array detected a
379 wide array of MGEs including insertional sequences, integrases, transposases and
380 plasmids mostly in food waste but also in digestates. Class 1 integron-integrase gene *intI1*
381 has been suggested as a proxy for anthropogenic pollution (66) and was also found in
382 digester feed (in the range of 10^5–10^6 copies/g-dw) as well as in digestion products (10^8
383 copies/g-dw) of this study. The occurrence of *intI1* in digestion products correlates with
384 the limited number of ARGs detected in the digestates of this study, showing that
385 anthropogenic impact remains detectable after AD.

386 **Potential bacterial host organisms of ARGs.** Correlation and network analysis
387 have been used in previous studies to link ARGs to their potential host organisms (35–37,
388 67). Here we used a combination of metagenomic assembly and binning methods to
389 detect taxonomically annotated contigs and MAGs with ARGs.

390 Taxonomic annotation of ARG-containing contigs showed several connections
391 between bacterial genera and ARGs. Among others, the genera *Enterobacter*, *Klebsiella*,
392 *Acinetobacter* and *Pseudomonas* that include medically critical ESKAPE-pathogens were
393 connected to ARGs in digester feed conferring resistance to multidrug, MLS, polymyxin,
394 aminoglycoside, triclosan and bacitracin antibiotics. The high number of multidrug
395 resistance genes is especially worrisome as these genes have the potential to confer

resistance to several types of antibiotics. Besides that, 12 out of 14 of the most abundant ARG-containing genera in digester feed also included resistance genes to polymyxin, a last-resort antibiotic used against Gram-negative bacteria. This highlights the spread of ARGs against last-resort antibiotics among various bacterial genera. ARG-containing genera that include clinically important species were also detected in digestion products, including *Burkholderia*, *Arcobacter*, *Streptococcus* and *Escherichia* indicating the risks associated with the possible use of anaerobic digestates.

Using metagenomic binning we were able to detect four ARG-containing MAGs originating from digester feed. A MAG annotated as *Erwinia* was found to carry 19 ARGs conferring resistance to several classes of antibiotics. Although *Erwinia* is commonly known as a plant pathogen, few cases of human infections have also been reported (68, 69). As *Erwinia* is closely related to other *Enterobacteriaceae* that include several known human pathogens, horizontal transfer of ARGs from plant-pathogenic *Erwinia* to human pathogenic genera may occur. Additionally, MAGs annotated as lactic acid bacteria *Lactococcus lactis* and *Lactobacillus* were found to carry ARGs. These bacteria are extensively used for food fermentation processes and are often regarded as probiotics, however, increasing evidence suggests they are reservoirs of potentially transmissible ARGs and may play a crucial role in the acquisition of AMR via food (70). It is noteworthy, that although 75% of all MAGs originated from digestion products, these MAGs did not contain ARGs. This correlates with the low abundance of ARGs in digestion products detected by other methods used in this study.

Methodological aspects of ARG detection. In this study total community metagenome data and HT-qPCR data were used to characterize the diversity and

419 abundance of ARGs. While the metagenomic approach has the potential to detect higher
420 diversity of ARGs due to large reference databases, qPCR of ARGs, although being
421 limited in the number of ARGs even in high-throughput applications, can provide higher
422 sensitivity (71). Our results showed that both metagenomic and HT-qPCR approach
423 detected a broad spectrum of ARGs with higher diversity of ARGs in digester feed
424 compared to digestion products. Among the limited number of ARGs detected in
425 digestion products *mphA*, *lnuB*, *catQ* and *tet44* were found to be unique to digestate by
426 both metagenomic and HT-qPCR approach. Additionally, HT-qPCR provided absolute
427 abundance values for individual ARGs that could be related back to the number of gene
428 copies present per gram of digester feed and in digested solids. This type of quantitative
429 information on ARGs is required for assessing the risks associated with food waste and
430 the use of anaerobic digestates as agricultural fertilizers. Thus, a combination of shotgun
431 metagenomic and qPCR approach is recommended for a comprehensive view of a
432 sample's resistome and for the assessment of AMR risks.

433 In addition to the annotation of short sequencing reads, we used assembled
434 contigs to provide genomic context of ARGs. The distribution of ARG types on
435 assembled contigs closely resembled that on short sequencing reads, additionally,
436 assembled data provided information about genomic location and potential host
437 organisms of ARGs, which cannot be determined from short sequencing reads. Although
438 assembled contigs provide better resolution for taxonomic and functional annotation than
439 short sequencing reads, assembly may also introduce biases (71). Similar problem has
440 been noted for metagenomic binning, which often has limited power for analysis of
441 complex microbial communities. This was also evident in the results of the current study

with 75% of MAGs (151 MAGs) originating from digestion products and only 25% of MAGs (50 MAGs) originating from the heterogeneous microbial communities of digester feed. Thus, our findings should be interpreted in light of the limitations of current methods used for taxonomic and functional analysis of complex microbial communities.

In conclusion, using a combination of metagenome sequencing, assembly and binning as well as quantitative PCR analysis is recommended to estimate the diversity and abundance of ARGs and to situate the ARGs within their genomic context and potential host organisms. The detection of potential host organisms and genomic context allows for a more accurate risk evaluation associated with solid organic wastes.

MATERIALS AND METHODS

Anaerobic digestion system and feedstock. A lab-scale anaerobic digestion system designed for the treatment of solid organic waste was operated for a total of 88 weeks. System design, its operating parameters and feedstock have been described in detail by Guilford *et al*. (40). In short, the anaerobic digestion system consisted of 6 leach beds (8.5 L each), an upflow anaerobic sludge blanket reactor (UASB, 27.5 L) treating leach bed leachate, a UASB feed tank (17.5 L), a leach bed feed tank (17.5 L), three peristaltic pumps to recirculate leachate, two wet-tip gas meters for biogas measurement, and an automated control system. The system was maintained at 37–39°C with continuous recirculation of the leachate. It was operated in sequential batch feeding mode: each week one of the leach beds was filled with a mixture of lignocellulosic fibres and food waste (FW) making up the leach bed feed (LBF), recovered from local residential waste recycling programs. Lignocellulosic fibres included shredded cardboard,

boxboard, fine paper and newsprint; food waste was collected from a residential green bin program and sorted manually to remove bones and inorganic materials. Solids retention time in the system was six weeks.

Sampling and DNA extraction. Samples for microbial community analysis were collected during weeks 75–84 of the experiment, during which FW contributed 21.3% of the total COD in the feedstock and the system exhibited stable performance with a methane yield of 246 L-CH_4 kg-VS_{added}^{-1} and a substrate destruction efficiency, as volatile solids (VS), of 63.5% (40). Ten 50 mL samples were collected: three food waste samples FW1, FW2, FW3 (FW source and composition has been described by Guilford *et al*. (40)); three leach bed feed samples LBF1, LBF2, LBF3 consisting of the respective food waste mixed with lignocellulosic fibres; three digestate samples DG74, DG76, DG78 collected after 6 weeks of digestion; and one sample from the microbial granular sludge of the UASB reactor (UASB). It should be noted that DG74, DG76 and DG78 were not direct digestion products of LBF1, LBF2 and LBF3, respectively, although derived from the same source of FW and lignocellulosic fibres used for LBF1, LBF2 and LBF3. All samples were preserved at –20°C until DNA extraction.

Total community DNA was extracted using the PowerMax Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) from 5 g of sample according to manufacturer's protocol. FW and LBF samples were further purified with 5 M NaCl and 100% ethanol as recommended by the manufacturer. The quantity and quality of DNA extracts was confirmed using NanoDrop spectrophotometer ND-1000 (Wilmington, DE, USA). All DNA extracts were stored at –80°C.

Quantitative PCR analyses. Quantitative PCR (qPCR) was used to quantify total bacterial (method 1.1 in Fig. 1) and archaeal (method 1.2 in Fig. 1) 16S rRNA gene copies using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) and previously published primers targeting most bacterial 16S rRNA genes: Bac1055f (5'-ATGGCTGTCGTCAGCT-3') and Bac1392r (5'-ACGGGCGGTGTGTAC-3') (72, 73), and most archaeal 16S rRNA genes: Arch787f (5'-ATTAGATACCCGBGTAGTCC-3') and Arch1059r (5'-GCCATGCACCWCCTCT-3') (74). qPCR reactions were performed in 20 µL comprising 10 µL SsoFast EvaGreen Supermix (Bio-Rad Laboratories, Hercules, CA, USA), 0.5 µM each forward and reverse primer, 2 µL template DNA, and sterile UltraPure distilled water. The thermocycling program was as follows: initial denaturation at 98°C for 2 min, followed by 40 cycles of denaturation at 98°C for 5 s, annealing at Tm (55°C for bacterial 16S rRNA, 60°C for archaeal 16S rRNA) for 10 s, and final melting curve analysis in the range of 65–95°C (steps of 0.5°C for 5 s). All samples were measured in three technical replicates and every qPCR run included negative controls containing all reaction components except for template DNA. Serial dilutions of plasmid stocks containing corresponding 16S rRNA gene fragments were used to generate standard curves. Amplification data was analyzed using CFX Manager Software v.3.1. Copy numbers of bacterial and archaeal 16S rRNA genes per gram of sample dry weight were calculated as follows: AbsoluteAbundance = StartingQuantity * DNAElutionVolume / (SampleWeight*SampleDW) where StartingQuantity represents the gene copies in 1 µL DNA extract as estimated by the CFX Manager Software, DNAElutionVolume was 5000 µL for DG74, DG76, DG78, UASB and 100 µL for FW1, FW2, FW3, LBF1, LBF2, LBF3, SampleWeight was the

510 mass of the sample used for DNA extraction and SampleDW was the dry weight of the
511 sample material used for DNA extraction.

512 A high-throughput qPCR (HT-qPCR) array was used for the quantification of
513 ARGs (method 4.1 in Fig. 1) and MGEs (method 4.2 in Fig. 1) in FW1, FW2, DG74 and
514 DG78. DNA extracts were sent to Dr Robert Stedtfeld at Michigan State University
515 (USA) who performed the analysis. A total of 384 primer sets were used targeting 315
516 ARGs and 57 MGEs, additionally taxonomic marker genes were included in the array
517 (75). All HT-qPCR reactions were performed using Takara (previously WaferGen)
518 SmartChip real-time PCR system as described previously (76). In brief, 5184 parallel
519 qPCR reactions (100 nL) were dispensed into the SmartChip using the SmartChip
520 Multisample Nanodispenser, followed by thermal cycling in the SmartChip Cycler,
521 automatic melting process and initial data processing with SmartChip qPCR Software
522 (v.2.7.0.1) as described before (76). Wells with multiple melt peaks and wells with
523 amplification efficiency outside the range 1.75 to 2.25 were removed from analysis. Each
524 sample was analyzed by three technical replicates. Genes detected in only one of the
525 replicates were considered false positives and removed from analysis. Genomic copy
526 numbers were estimated using the formula in Looft *et al.* (77) with the exception of
527 setting detection limit to threshold cycle (Ct) 28 as suggested by Stedtfeld *et al.* (75).
528 Relative abundances of the studied genes were calculated as ratios to 16S rRNA:
529 $RelativeAbundance_{gene} = GenomicCopyNumber_{gene} / GenomicCopyNumber_{16S}$. Absolute
530 abundances were determined by multiplying the relative abundance of a gene by 16S
531 rRNA absolute abundance (determined by regular qPCR analysis described above):
532 $AbsoluteAbundance_{gene} = RelativeAbundance_{gene} * AbsoluteAbundance_{16S}$. ARG

533 absolute abundances were visualized in R software using the package ComplexHeatmap
534 (78).

535 **SSU rRNA gene amplicon sequencing and taxonomic analyses.** SSU rRNA
536 gene sequencing with the universal primers 926f-modified (5'-
537 AAACTYAAAKGAATWGRCGG-3') and 1392r-modified (5'-
538 ACGGGCGGTGWGTRC-3') (modified from (79)) targeting the V6–V8 variable region
539 of the 16S rRNA gene from bacteria and archaea as well as the 18S rRNA gene in
540 eukarya was performed at McGill University and Génome Québec Innovation Centre
541 (Canada) on Illumina MiSeq System (PE300). The analysis of sequencing reads was
542 performed with QIIME1 package (tool 2.1 in Fig. 1) (80) by joining forward and reverse
543 reads (multiple_join_paired_ends.py, min overlap 50 bp, max mismatch allowance 8),
544 removing low-quality sequences (multiple_split_libraries_fastq.py, quality threshold 19),
545 removing chimeric sequences and identifying operational taxonomic units at 97%
546 similarity (identify_chimeric_seqs.py, pick_open_reference_otus.py, method usearch61
547 (81), reference database SILVA release 128).

548 **Total community metagenome sequencing and bioinformatic workflow.** Total
549 community metagenome sequencing of the samples FW1, FW2, LBF1, LBF2, DG74,
550 DG78, UASB was performed at University of Toronto Centre for the Analysis of
551 Genome Evolution and Function (Canada) on Illumina NextSeq500 Desktop Sequencer
552 v2 using High Output flowcell (PE150). Bioinformatic workflow included quality control
553 and trimming of the sequencing reads, taxonomic classification, metagenomic assembly
554 and binning, and detection of ARGs. Sequence reads were trimmed with Trimmomatic
555 v.0.32 (82) (tool 3.1 in Fig. 1) using default settings for paired-end mode, additional

556 trimming to remove polyG sequences (≥30 bp) was performed with AfterQC v.0.9.1 (83)
557 (tool 3.2 in Fig. 1).

558 Quality-trimmed reads were subjected to taxonomic classification using Kaiju
559 v.1.4.5 (84) (tool 3.3 in Fig. 1) in greedy mode (allowed substitutions 5, minimum
560 required match length 11, minimum required match score 70) with bacterial, archaeal,
561 eukaryotic and viral protein sequences from the NCBI nr database as reference. To
562 further investigate microbial community composition, metaxa2 v.2.1 (85) (tool 3.4 in Fig.
563 1) was used to extract small subunit rRNA gene reads in metagenome mode with default
564 search criteria and reliability score cut-off 80.

565 Quality-trimmed reads were assembled into longer DNA contigs using
566 MEGAHIT v.1.1.1 (86) (tool 3.6 in Fig. 1) and metaSPAdes v.3.10.1 (87) (tool 3.7 in
567 Fig. 1) with default settings. Assemblies were compared using metaQUAST v.4.5 (88)
568 which also provided assembly statistics. Metagenome binning was performed using
569 MaxBin v.2.2.3 (89) (tool 3.11 in Fig. 1) and MetaBAT v.2.12 (90) (tool 3.12 in Fig. 1)
570 with default settings on the MEGAHIT assemblies. All genome bins produced by both
571 binning methods were dereplicated using dRep v.1.4.3 (91) (tool 3.13 in Fig. 1) with
572 default settings (ANI cut-off 99%, minimum genome completeness 75%, maximum
573 contamination 25%) and evaluated with CheckM v.1.0.7 (92). Quality of the resulting
574 metagenome-assembled genomes (MAGs) was defined as (Completeness –
575 5*Contamination) as suggested by Parks *et al*. (93). MAGs with quality >50 and
576 taxonomic classification to at least phylum level, were submitted to the JGI Integrated
577 Microbial Genomes and Microbiomes (IMG) database for annotation. Anvi'o v.5 (94)
578 was used for visualizing MAGs clustered based on the relative abundance of MAGs in

579 the samples: Euclidian distances were calculated from relative abundance estimates of
580 each MAG and clustering was performed using the ward linkage algorithm. Relative
581 abundance of each MAG was calculated as number of reads recruited to the MAG
582 divided by total reads recruited to that MAG across all samples.

583 ARGs from subsets of quality-trimmed sequencing reads (20 million
584 reads/sample) were determined with ARGs-OAP v.1.2 (41) (tool 3.5 in Fig. 1) using the
585 default reference database SARG and default settings. The same reference database was
586 also used to detect ARGs on assembled contigs by local BLASTp (evalue 1e-7, identity
587 percent 80, minimal alignment length 25) using protein coding genes annotated by JGI
588 IMG/M 4 version (95) as query sequences (tool 3.10 in Fig. 1). Contigs with multiple
589 ARGs were identified by a local python script that counted how many times a contig ID
590 appeared in the list of contigs that carried ARGs (Method S1 in supplemental material).
591 PlasFlow v.1.1 (65) with default settings was used to predict plasmid sequences from the
592 contigs with ARGs (tool 3.8 in Fig. 1). Taxonomic classification of the non-plasmid
593 contigs carrying ARGs was determined with Kaiju v.1.4.5 (84) in greedy mode
594 (maximum mismatches allowed 5, minimal required match length 11, minimal required
595 match score 70, filtering of query sequences containing low-complexity regions on) (tool
596 3.9 in Fig. 1) using NCBI RefSeq reference database and visualized with Cytoscape
597 v.3.6.1 (96) by selecting 10 most abundant ARGs from all genera above relative
598 abundance of 1%. The presence of ARGs in MAGs was determined by a local python
599 script comparing a list of ARG-carrying contigs to the list of contigs included in the
600 MAG (tool 3.14 in Fig. 1, Method S2 in supplemental material).

Accession numbers. Sequencing data from SSU rRNA gene sequencing (accession numbers provided in Table S1 in supplemental material) and total community metagenome sequencing (accession numbers provided in Table S3 in supplemental material) is available on NCBI Sequence Read Archive under BioProject PRJNA501900, SRA study SRP167436, accession numbers SRX4965138–47 (SSU rRNA gene sequencing) and SRX4986160–6 (total community metagenome sequencing). Additionally, metagenome assemblies (accession numbers provided in Table S6 in supplemental material) and metagenome assembled genomes (accession numbers provided in Table S9 in supplemental material) have been deposited to the JGI Integrated Microbial Genomes and Microbiomes (https://img.jgi.doe.gov/cgi-bin/m/main.cgi).

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925 TABLES

926 **TABLE 1** Genomic location of antibiotic resistance genes (ARGs) detected in total
927 community metagenome sequencing data assembled with MEGAHIT. ARGs were
928 detected by BLAST analysis of protein coding genes using local SARG reference
929 database. Plasmid or chromosomal location of the ARGs was predicted by PlasFlow. All
930 ARG BLAST hits detected in MEGAHIT-assembled metagenome sequencing data are
931 provided in Table S7 in supplemental material.

932

Sample	Total assembly length (bp)	Protein coding genes	Number of ARG BLAST hits (% from protein coding genes)	Plasmid ARGs (%)	Chromosomal ARGs (%)
FW1	1 285 684 727	2 088 107	2 407 (0.12%)	30.2	28.6
FW2	941 870 975	1 796 492	2 484 (0.14%)	34.7	23.0
LBF1	120 537 563	1 821 640	1 908 (0.10%)	30.0	28.9
LBF2	1 044 915 087	1 906 512	1 938 (0.10%)	33.2	21.9
DG74	726 434 840	1 137 099	11 (0.001%)	36.4	9.1
DG78	729 045 788	1 138 132	24 (0.002%)	33.3	8.3
UASB	941 623 397	1 636 065	20 (0.001%)	60.0	10.0

933

934 **TABLE 2** Bacterial host organisms of non-plasmid ARGs detected in digestion products
935 (DG74, DG78, UASB). ARGs were identified on MEGAHIT-assembled contigs by
936 BLAST analysis of protein coding genes using local SARG reference database (Table S7
937 in supplemental material). Non-plasmid contigs carrying ARGs were further annotated
938 for their taxonomic affiliation using Kaiju.
939

Kingdom/Phylum/Class	Family/Genus	ARG	ARG type
Firmicutes	not determined	*ANT(6)-Ia*	aminoglycoside
Terrabacteria group	not determined	*ANT(9)-Ia*	aminoglycoside
Bacteroidetes	*Proteiniphilum*	*bacA*	bacitracin
Betaproteobacteria	*Thauera*	*bacA*	bacitracin
Deltaproteobacteria	*Desulfomicrobium*	*bacA*	bacitracin
Firmicutes	*Alkaliphilus*	*bacA*	bacitracin
Firmicutes	*Thermoanaerobacterium*	*bcrA*	bacitracin
Kiritimatiellaeota	*Kiritimatiella*	*OXA-119*	beta-lactam
Betaproteobacteria	*Burkholderia*	*katG*	isoniazid
Epsilonproteobacteria	*Arcobacter*	*katG*	isoniazid
Bacteroidetes	*Fermentimonas*	*ermF*	MLS
Firmicutes	not determined	*linB*	MLS
Deltaproteobacteria	*Desulfococcus*	*lnuF*	MLS
Deltaproteobacteria	*Geobacter*	*lnuF*	MLS
Firmicutes	*Streptococcus*	*mefA*	MLS
Gammaproteobacteria	*Escherichia*	*mefB**	MLS
Firmicutes	*Clostridium*	*mel*	MLS
Firmicutes	*Desulfitobacterium*	*vatB*	MLS
Betaproteobacteria	*Thauera*	*acrB*	multidrug
Deltaproteobacteria	*Desulfobacca*	*acrB*	multidrug
Deltaproteobacteria	*Syntrophobacter*	*acrB**	multidrug
Planctomycetes	*Thermogutta*	*acrB*	multidrug
Firmicutes	not determined	*lsaE*	multidrug
Deltaproteobacteria	*Desulfobacca*	*smeE*	multidrug
Gammaproteobacteria	*Sedimenticola*	*smeR*	multidrug
Betaproteobacteria	*Roseateles*	*arnA*	polymyxin
Gammaproteobacteria	*Enterobacteriaceae*	*arnA*	polymyxin
Bacteria	not determined	*sul1*	sulfonamide
Bacteria	not determined	*sul1*	sulfonamide

940 * ARG was affiliated to the respective genus on several contigs
941

TABLE 3 Metagenome assembled genomes (MAGs) containing antibiotic resistance genes (ARGs) originating from digester feed (FW1, FW2, LBF1, LBF2). ARGs were not detected in MAGs from digestion products. MAGs were produced by MaxBin and metaBAT, dereplicated with dRep and evaluated with CheckM for genome size, GC%, completeness, contamination and estimated quality. ARGs that were detected in two separate copies in a given MAG are indicated by parenthesis (2x). All ARG-containing MAGs have been deposited to the JGI Integrated Microbial Genomes and Microbiomes (IMG/M). Additional information about all MAGs is available in Table S9 and Figure S4 in supplemental material.

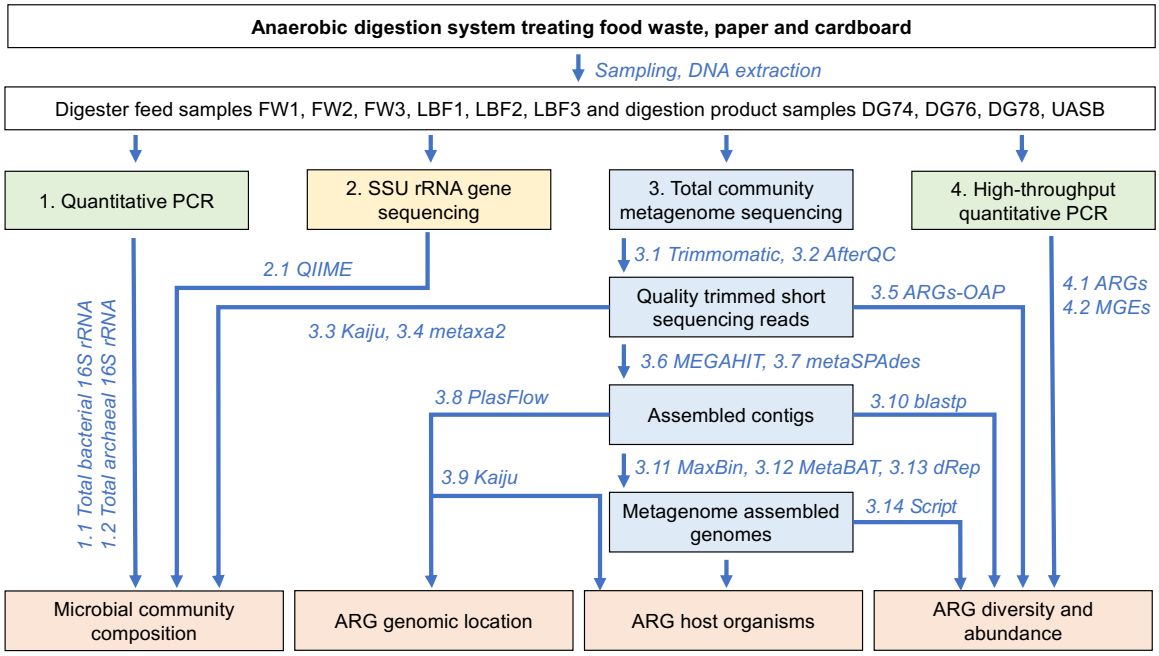
MAG	IMG/M ID	Genome size (bp)	GC%	Completeness (%)	Contamination (%)	Estimated quality	ARG	ARG type
Erwinia	2772190821	5 042 753	56.0	97.9	1.9	88.2	*acrD*	aminoglycoside
							bacA	bacitracin
							acrB	multidrug
							cpxA	multidrug
							cpxR (2x)	multidrug
							CRP	multidrug
							emrR	multidrug
							H-NS	multidrug
							mdtB (2x)	multidrug
							mdtC	multidrug
							arnA	polymyxin
							phoP	polymyxin
							pmrF	polymyxin
							rosA	polymyxin

							rosB	polymyxin
							emrB	quinolone
							folP	sulfonamide
	Lactococcus lactis	2773857626	3 416 378	33.7	92.0	5.7	63.4	*lmrC*	MLS
							lmrD	MLS
							lmrP	multidrug
							tetM	tetracycline
							tetS	tetracycline
	Lactobacillus	2772190822	2 569 052	30.9	97.0	3.6	79.1	*dfrE*	trimethoprim
	Bifidobacteriaceae	2806310579	2 067 166	64.6	90.7	2.4	78.6	*mdsB*	multidrug

949

950 FIGURES

951



952

953 **FIG 1** Experimental workflow of anaerobic digestion experiment. Total community

954 DNA extracts from 10 samples including food waste (FW1, FW2, FW3), leach bed feed

955 (LBF1, LBF2, LBF3), digestate (DG74, DG76, DG78) and microbial granules from

956 UASB reactor (UASB) were subjected to small subunit (SSU) rRNA gene amplicon

957 sequencing (2), total community metagenome sequencing (3) and quantitative PCR

958 analysis (1, 4). Sequencing data was analyzed using a variety of bioinformatic tools

959 shown and numbered in italics. SSU rRNA gene sequencing data was analyzed using

960 QIIME1 (tool 2.1). The workflow for total community metagenome sequencing data

961 included quality trimming of the short sequencing reads (tools 3.1 and 3.2), taxonomic

962 annotation of the quality trimmed reads (tools 3.3 and 3.4), detection of ARGs from

963 quality trimmed reads (tool 3.5), assembly of the quality trimmed reads into contigs

964 (tools 3.6 and 3.7), identification of plasmid sequences (tool 3.8) and ARGs (tool 3.10)

965 from assembled contigs, taxonomic annotation of assembled contigs (tool 3.9), binning of
966 the assembled contigs into metagenome assembled genomes (tools 3.11, 3.12, 3.13) and
967 detection of ARGs in metagenome assembled genomes by a local Python script (tool
968 3.14). Quantitative PCR was performed for bacterial (1.1) and archaeal (1.2) 16S rRNA
969 gene as well as for ARGs (4.1) and MGEs (4.2) using a high-throughput qPCR array.
970 Results covered microbial community composition, ARG diversity, abundance, genomic
971 location and potential bacterial host organisms of ARGs.
972

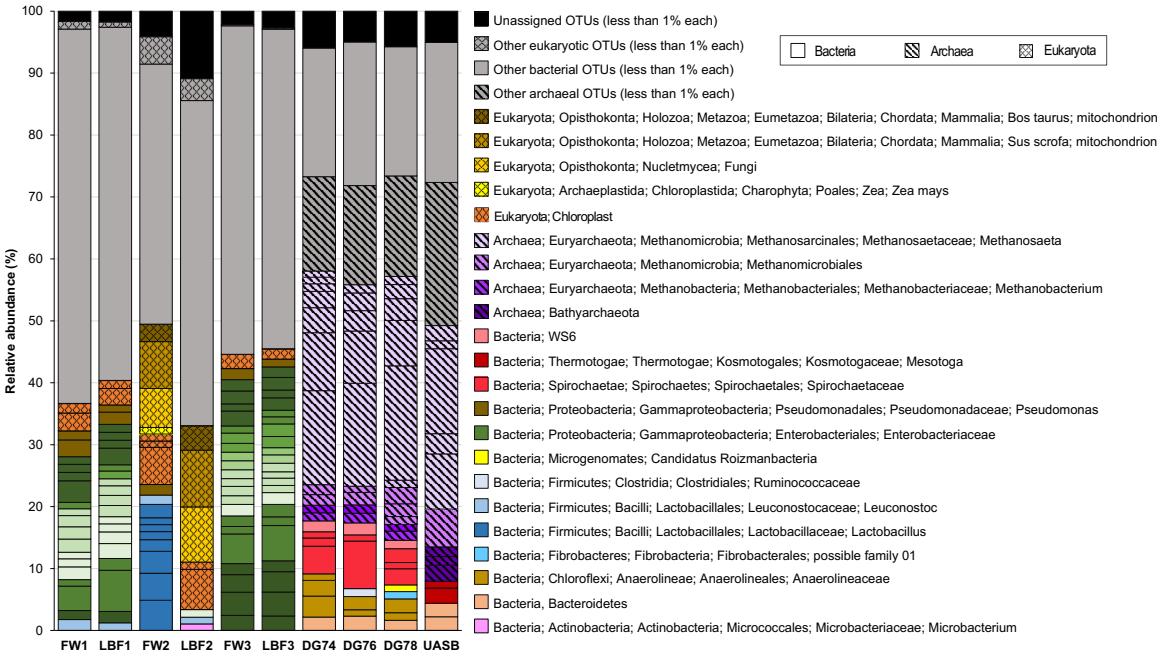
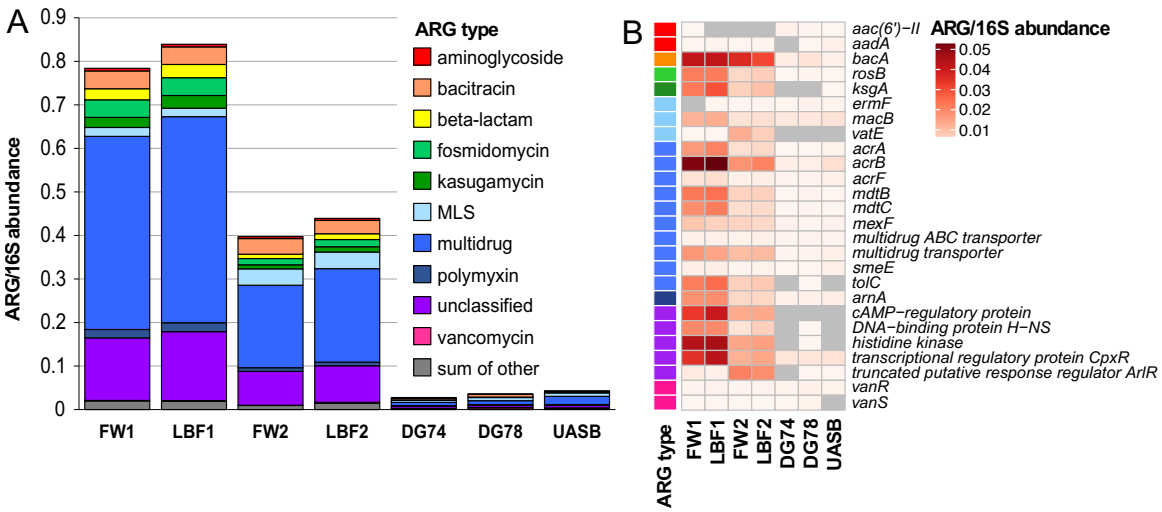


FIG 2 Taxonomic composition of bacterial, archaeal and eukaryotic OTUs detected by SSU rRNA gene sequencing. Each rectangle represents an individual OTU with relative abundance ≥1%. The remaining OTUs with relative abundance <1% are summed and represented in grey as separate rectangles for bacteria, archaea and eukaryota. Bacterial OTUs are presented in solid colors, archaeal OTUs with vertical lines and eukaryotic OTUs in diamond pattern. OTUs belonging to the bacterial family *Enterobacteriaceae* are presented in various shades of green. Additional information on all detected OTUs is provided in Table S2 in supplemental material.



983

984 **FIG 3** Relative abundance of ARGs per 16S rRNA gene categorized by type (A) and by

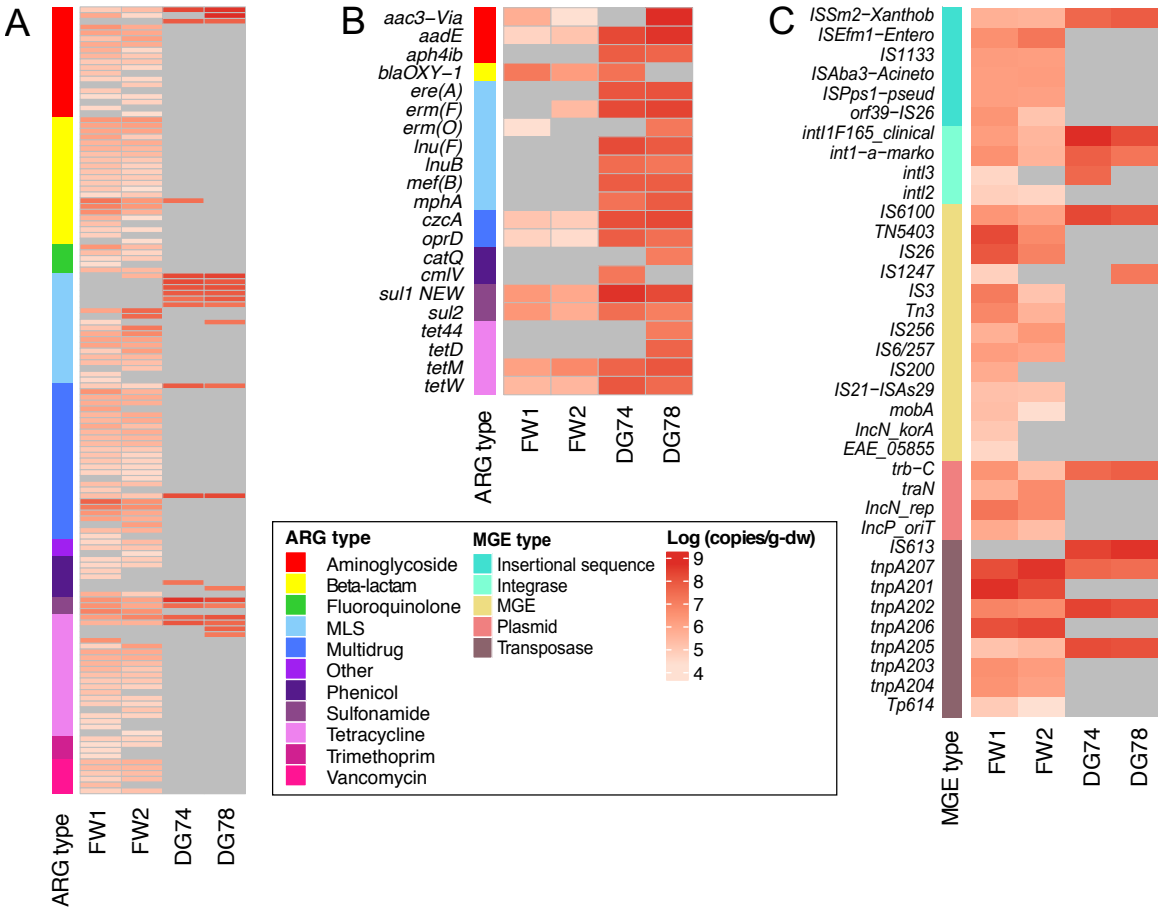
985 gene (B) detected in total community metagenome sequencing reads using ARGs-OAP.

986 The ten most abundant ARGs from each sample are depicted in panel B. Genes not

987 detected are marked in grey. Relative abundances of all detected ARGs are provided in

988 Table S4 in supplemental material.

989



990

991 **FIG 4** Absolute abundance of ARGs and MGEs in food waste (FW1, FW2) and digestate

992 (DG74, DG78) samples detected using a high-throughput qPCR array. Absolute

993 abundance of all detected genes is presented on log-scale as gene copies per gram of dry

994 weight of food waste or digestate samples. Genes not detected are marked in grey. Panel

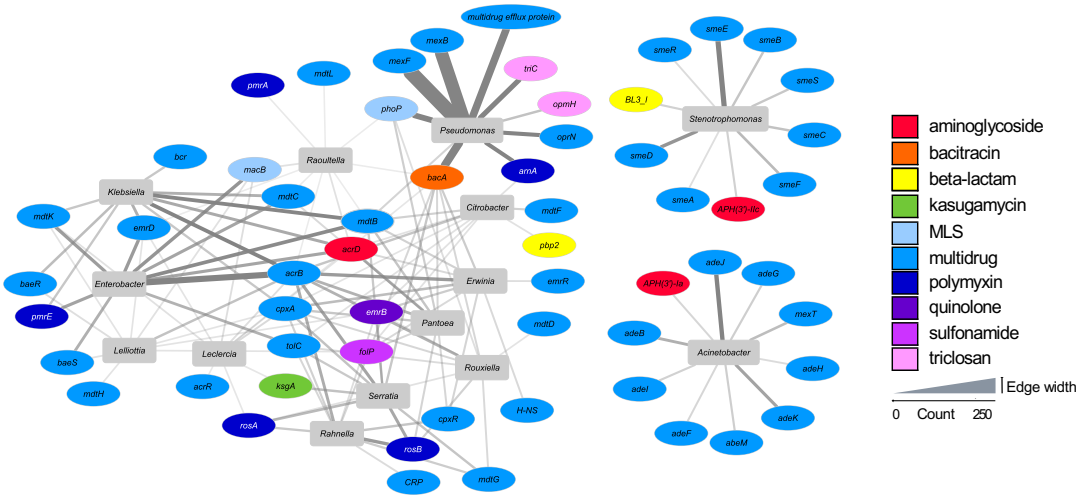
995 A shows all ARGs detected in at least one of the four samples. Panel B shows all ARGs

996 detected in at least one of the digestate samples. Panel C shows all MGEs detected in at

997 least one of the four samples. Raw data for all analyzed genes together with abundance

998 calculations are provided in Table S5 in supplemental material.

999



1000

1001 **FIG 5** Most abundant bacterial host genera of non-plasmid ARGs detected in digester

1002 feed (FW1, FW2, LBF1, LBF2). ARGs were identified on MEGAHIT-assembled contigs

1003 by BLAST analysis of protein coding genes using local SARG reference database (Table

1004 S7 in supplemental material). Non-plasmid contigs carrying ARGs were further

1005 annotated for their taxonomic affiliation using Kaiju. ARGs are depicted in ellipses

1006 colored by resistance type and bacterial genera are depicted in grey rectangles. The width

1007 of the connecting edge corresponds to detection frequency of individual host-ARG pairs.