

1 **Plastic Leachate Exposure Drives Antibiotic Resistance and Virulence in Marine**

2 **Bacterial Communities**

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19

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21

22 **Abstract**

23

24 Plastic pollution is a serious global problem, with more than 12 million tonnes of plastic
25 waste entering the oceans every year. Plastic debris can have considerable impacts on
26 microbial community structure and functions in marine environments, and has been
27 associated with an enrichment in pathogenic bacteria and antimicrobial resistance (AMR)
28 genes. However, our understanding of these impacts is largely restricted to microbial
29 assemblages on plastic surfaces. It is therefore unclear whether these effects are driven by the
30 surface properties of plastics, providing an additional niche for certain microbes residing in
31 biofilms, and/or chemicals leached from plastics, the effects of which could extend to
32 surrounding planktonic bacteria. Here, we examine the effects of polyvinyl chloride (PVC)
33 plastic leachate exposure on the relative abundance of genes associated with bacterial
34 pathogenicity and AMR within a seawater microcosm community. We show that PVC
35 leachate, in the absence of plastic surfaces, drives an enrichment in AMR and virulence
36 genes. In particular, leachate exposure significantly enriches AMR genes that confer
37 multidrug, aminoglycoside and peptide antibiotic resistance. Additionally, enrichment of
38 genes involved in the extracellular secretion of virulence proteins was observed among
39 pathogens of marine organisms. This study provides the first evidence that chemicals leached
40 from plastic particles alone can enrich genes related to microbial pathogenesis within a
41 bacterial community, expanding our knowledge of the environmental impacts of plastic
42 pollution with potential consequences for human and ecosystem health.

43 **1. Introduction**

44

45 Plastic pollution in marine ecosystems has become a serious global problem, with more than
46 12 million metric tonnes of plastic waste ending up in the oceans every year (Borrelle et al.,
47 2020; Jambeck et al., 2015; Lau et al., 2020). As plastic production rates continue to rise and
48 poor waste management practices remain in many areas of the world, issues associated with
49 marine plastic pollution are likely to increase in the future (Borrelle et al., 2020; Jadhav et al.,
50 2022; Lau et al., 2020; Lebreton and Andrady, 2019). To date, recognition of the
51 environmental impacts of plastic debris has largely focused on entanglement and ingestion by
52 marine species (Cózar et al., 2014; Gregory, 2009; Lebreton et al., 2018; Wright et al., 2013).
53 However, additional impacts are increasingly being recognised for marine microorganisms.
54 These include the environmental release of chemicals via leaching from plastic particles,
55 which can significantly alter marine microbial communities (Capolupo et al., 2020; Focardi et
56 al., 2022; Gunaalan et al., 2020; Romera-Castillo et al., 2018) and dissemination of
57 pathogenic microorganisms, via rafting on plastic debris (Bhagwat et al., 2021; Bryant et al.,
58 2016; Zettler et al., 2013; Zhang et al., 2022).

59 Plastics are known to leach a variety of organic and inorganic substances through
60 weathering and biological degradation processes. This includes additives such as plasticizers,
61 UV stabilizers, metals, and dyes, most of which are not chemically bound to the polymer
62 matrix (Hahladakis et al., 2018; Hermabessiere et al., 2017). Some of these additives are
63 known endocrine-disrupters, reproductive toxicants, carcinogens, and mutagens (Wiesinger et
64 al., 2021; Zimmermann et al., 2019). The ability to tolerate exposure to common inorganic
65 and/or organic components of plastic leachate has been shown to be highly variable across
66 different marine microbes. Exposure to chemicals leaching from plastics is detrimental to
67 some marine organisms, such as zooplankton (Gewert et al., 2021; Lithner et al., 2009), green

68 algae (Simon et al., 2021), bacterial picocyanobacteria (Sarker et al., 2020; Tetu et al., 2019),
69 and other keystone marine microbes, including SAR11 (Focardi et al., 2022). However, some
70 marine heterotrophic bacteria appear to benefit from plastic leach exposure, likely from the
71 increase in available dissolved organic carbon (Birstiel et al., 2022; Focardi et al., 2022;
72 Romera-Castillo et al., 2018).

73 Colonisation of plastic debris by microorganisms, termed the “plastisphere”, has been
74 extensively studied, with clear indications that this niche selects for microbial communities
75 differing in abundance and diversity from the surrounding waters (Bryant et al., 2016;
76 Dussud et al., 2018; He et al., 2022; Zettler et al., 2013). Of particular concern is the
77 enrichment of potential pathogens and antibiotic resistant microbes on plastic particles, as
78 well as increases in antimicrobial resistance (AMR) genes (Di Pippo et al., 2022; Loiseau and
79 Sorci, 2022; Oberbeckmann et al., 2015; Sathicq et al., 2021; Sucato et al., 2021; Sun et al.,
80 2021; Wang et al., 2020; Yang et al., 2019; Zhang et al., 2022). Studies using metagenomics-
81 derived data have found higher relative abundance, diversity and richness indices of human
82 and fish pathogens in the microbial communities attached to plastics in the Mediterranean
83 Sea (Dussud et al., 2018), the North Pacific Gyre (Yang et al., 2019), and in coastal regions
84 of Norway (Radisic et al., 2020), the Gulf of Mexico (Sun et al., 2021), and Eastern Australia
85 (Bhagwat et al., 2021).

86 It is clear that marine plastic debris has the potential to serve as a vector for pathogens
87 and genes involved in virulence and antibiotic resistance. However, it is not clear whether
88 this is due solely to plastic debris providing an additional niche for certain microbes,
89 particularly those residing in biofilms, or because leached plastic chemicals also favour
90 increases in such microorganisms. To our knowledge there have been no studies examining
91 whether chemicals that leach from plastic waste select for higher relative abundances of
92 AMR and pathogenicity traits within a community, independent of the physical effects linked

93 to plastic particles. Here we demonstrate that polyvinyl chloride (PVC) plastic leachate
94 enriches for virulence and AMR genes in a marine microbial community from Eastern
95 Australian coastal shelf waters (Focardi et al., 2022).

96

97 **2. Methods**

98

99 *2.1. Data acquisition*

100

101 Metagenomic data was obtained from our previous study examining the effects of PVC
102 leachate and zinc, an abundant PVC additive, on a seawater microcosm community (Focardi
103 et al., 2022). Methodology for the leachate preparation, and microcosm experiment set-up has
104 been described in our previous study (Focardi et al., 2022). Briefly, microcosm samples were
105 subject to a six-day exposure to either 1% (0.5g/L) PVC leachate (PVC1), 10% (5g/L) PVC
106 leachate (PVC10), 0.13 mg/L zinc chloride (ZnL), 1.3 mg/mL zinc chloride (ZnH) (zinc
107 being the most abundant inorganic component in leachate from this PVC plastic), or left
108 untreated as control samples (SW). DNA extracted from each treatment was used to generate
109 metagenomic libraries at the Ramaciotti Center for Genomics (Sydney, Australia) using the
110 Illumina Nextera DNA Flex library preparation kit, and sequenced on the NovaSeq6000
111 platform (2x150 bp High Output run). Gene sequences, de-replicated at 98% nucleotide
112 identity, and gene counts for each sample were retrieved from Focardi et al. (2022). Detailed
113 methodology for the metagenomic data processing, assembly, gene prediction, and gene
114 counts has been described in our previous study. All raw sequence data are available under
115 NCBI BioProject accession PRJNA756323.

116

117 *2.2. Identification and quantification of AMR, virulence, and toxin genes*

118

119 AMR and pathogenicity-related genes from each sample were identified using PathoFact v1.0
120 (de Nies et al., 2021) with default settings. PathoFact is a pipeline that identifies putative
121 virulence factors, bacterial toxins, and AMR genes. PathoFact further classifies AMR genes
122 by antimicrobial category and resistance mechanism. For cases where AMR genes were
123 assigned multiple resistance mechanisms, we used only the first predicted mechanism from
124 PathoFact.

125 Relative abundance of each gene was estimated with normalised read counts using the
126 simplified transcripts per million (TPM) method described by Wagner et al. (2012). The per-
127 sample mean number of nucleotides mapped per feature was taken as a proxy for read length.
128 One-way ANOVAs followed by post-hoc Tukey-HSD tests were performed to compare the
129 relative abundance of genes in PVC treatment samples (PVC1 and PVC10) and Zn treatment
130 samples (ZnL and ZnH) independently against the control samples (SW) in each of the
131 PathoFact output categories (AMR, Virulence, and Toxin).

132

133 *2.3. Additional screening and categorisation of virulence genes*

134

135 PVC leachate treatment resulted in increases in the relative abundance of virulence genes that
136 were deemed significant, but were close to the significance cut-off (P-value < 0.05) as
137 predicted by PathoFact. Thus, to investigate this further, we used a second analysis pipeline
138 SeqScreen v3.4 (Balaji et al., 2022) with the SeqScreenDB v2.0 (version February 2022) to
139 test whether increases in the relative abundance of virulence genes were significant.
140 SeqScreen utilises a large set of curated Functions of Sequences of Concern (FunSoCs)
141 specific to microbial pathogenesis to both identify and characterise virulence genes. We
142 employed SeqScreen [parameters: --splitby 50000 --threads 24] to re-screen all metagenomic

143 data for virulence genes and determine which virulence functional categories these genes
144 were assigned to. Since our goal in using SeqScreen was specifically to identify bacterial
145 virulence factors, genes assigned to virus-specific categories or AMR were removed from the
146 SeqScreen output. One-way ANOVAs followed by post-hoc Tukey-HSD tests were
147 performed to compare the relative abundance of virulence genes among all treatment and
148 control groups.

149

150 *2.4. Enrichment of specific AMR/virulence categories and genes*

151

152 For PathoFact-predicted AMR genes, we compared the difference in relative abundance of
153 antimicrobial categories and resistance mechanisms between PVC treatments and control
154 samples. For SeqScreen-predicted virulence genes, we compared the difference in relative
155 abundance of bacterial virulence FunSoC categories. Categories which had a mean relative
156 abundance across PVC and seawater samples below 100 TPM for virulence and 10 TPM for
157 AMR were excluded from the results as we considered them unlikely to be biologically
158 relevant. For all comparisons, normalised gene counts were summed by category for each
159 sample. One-way ANOVA tests were run for each category and followed by post-hoc Tukey-
160 HSD tests where significant results were identified.

161 The set of predicted AMR genes (PathoFact) and virulence genes (SeqScreen) most
162 highly enriched among PVC leachate-treated communities were then identified for further
163 analysis. The genes most enriched in the PVC10 treatment compared to the seawater control
164 were identified by calculating the log₂-fold change of genes with a minimum mean relative
165 abundance of 9 TPM for AMR, and 20 TPM for virulence in the treatment group (in order to
166 select genes that were both highly abundant and highly enriched). We assigned putative
167 taxonomy to the 20 most highly enriched AMR genes using a BLASTn search against the

168 NCBI nt database. For virulence genes, we used the taxonomic assignments provided by
169 SeqScreen, which runs both a DIAMOND (Buchfink et al., 2015) search against a curated
170 UniRef100 database (Suzek et al., 2007), as well as running Centrifuge (Kim et al., 2016)
171 against Archaeal and Bacterial RefSeq genomes.

172

173 *2.5. Diversity of AMR and virulence genes*

174

175 Beta-diversity of AMR (PathoFact) and virulence (SeqScreen) gene profiles between treated
176 and untreated microbial communities were visualised using a non-metric multi-dimensional
177 scaling (nMDS) plot based on the Bray-Curtis index of normalised read counts. This was
178 achieved using the *vegdist* and *metaMDS* functions of the VEGAN v2.5-7 package (Oksanen
179 et al., 2013) in R (v 4.1.2). Significant differences between treatment groups were analysed
180 using multivariate PERMANOVA using the *adonis2* function in VEGAN.

181 Alpha diversity was calculated in R using Shannon-Weiner and Simpson indexes for
182 the AMR (PathoFact) and virulence (SeqScreen) gene sets. One-way ANOVA and post-hoc
183 Tukey-HSD tests were run against the resulting values across PVC1, PVC10 and SW.

184

185 **3. Results and Discussion**

186

187 Previously, we examined the effects of exposure to two concentrations of PVC plastic
188 leachate and of zinc, the most abundant inorganic PVC additive, on a marine microbial
189 community via a six-day microcosm experiment, showing that this leads to substantial
190 changes in community composition and function (Focardi et al., 2022). In this study, we have
191 analysed the metagenomic data from Focardi et al. (2022), to investigate whether exposure to

192 PVC leachate and/or zinc results in significant enrichment of genes associated with
193 pathogenicity and drug resistance.

194

195 *3.1. Plastic leachate exposure increases antibiotic resistance and virulence genes in marine*
196 *microbial communities*

197

198 Marine microbial communities treated with PVC leachate, in the absence of physical plastic
199 surfaces, showed a concentration-dependent increase in the relative abundance of AMR,
200 virulence and toxin genes compared to non-treated controls, although the increase in toxin
201 genes was not statistically significant (ANOVA, $p=0.082$, Suppl. Table 1f) (Fig 1a-c, based
202 on PathoFact predictions). Past analyses of leachate from this specific PVC plastic showed it
203 is comprised of a complex mix of both organic and inorganic substances, with levels of zinc,
204 a common PVC additive, found to be particularly high (Tetu et al., 2019). As Zn exposure
205 has previously been shown to increase the prevalence of antibiotic resistant bacteria in the
206 environment (e.g., Poole, 2017; Silva et al., 2021), and promote virulence in host-associated
207 bacteria (Wu et al., 2021), we also looked to see if exposure to zinc alone was sufficient to
208 account for the PVC leachate impact on AMR and virulence gene prevalence. Using
209 PathoFact predictions, we found that treatment with two concentrations of zinc, ZnL (0.13
210 mg/L ZnCl) and ZnH (1.3 mg/mL ZnCl), had no effect on the relative abundance of AMR,
211 virulence or toxicity genes compared to untreated seawater controls (Fig. 1d-f). This suggests
212 that zinc additives alone are not driving the observed effects of PVC leachate on AMR and
213 virulence gene enrichment.

214 AMR gene relative abundance showed a slight, non-significant increase in the 1%
215 PVC leachate treatments and a strong, significant increase in the 10% PVC leachate treatment
216 relative to untreated seawater controls (Fig. 1a; 2.4-fold increase; TukeyHSD, $p\text{-adj.}=0.009$,

217 Suppl. Table 1a, 1c). Similarly, for the set of virulence-associated genes based on PathoFact
218 predictions, 1% PVC leachate treatment resulted in a small non-significant increased while
219 the 10% PVC leachate treatment drove a significant increase in virulence genes (Fig. 1b; 1.2-
220 fold increase; TukeyHSD, p-adj.=0.049, Suppl. Table 1a, 1e). Given that this increase was
221 close to the significance cut-off, we performed further analysis of virulence genes, using the
222 recently developed SeqScreen pipeline which has a larger, curated virulence database (Balaji
223 et al., 2022). Based on this, both 1% and 10% PVC leachate treatments resulted in significant
224 enrichments of virulence genes (Suppl. Fig. 1, Tukey-HSD, p adj. = 0.022 and p adj. = 0.004,
225 respectively, Suppl. Table 5b), representing a 1.3-fold increase in virulence genes in the
226 PVC1 and 1.4-fold for PVC10 (Suppl. Table 5a).

227

228 *3.2. Plastic leachate exposure changes the makeup of AMR gene suites and resistance* 229 *mechanisms*

230

231 Both 1% and 10% PVC leachate treatments drove clear shifts in AMR gene profiles, evident
232 from non-metric multidimensional scaling (NMDS) analysis (Fig. 2a, stress value = 0.06,
233 indicating clear separation from both the control and zinc treatments) and supported by
234 PERMANOVA (p=0.001, $R^2 = 0.57$, Suppl. Table 2a). However, PVC treatments had no
235 significant effect on the alpha diversity of AMR genes (Fig. 2b, c), indicating that enrichment
236 of AMR genes following PVC leachate exposure is due to an increase in the relative
237 abundance of specific AMR genes, rather than an increase in overall AMR gene diversity.

238 The 10% PVC leachate treatment drove significant enrichments in several AMR
239 categories (Fig. 3a), including aminoglycoside (Tukey-HSD, p adj ≤ 0.0001), antimicrobial
240 peptide (Tukey-HSD, p adj=0.001), aminoglycoside:aminocoumarin (Tukey-HSD, p
241 adj=0.02), and multidrug resistance (Tukey-HSD, p adj=0.02). Aminoglycoside resistance

242 genes were also significantly enriched following the 1% PVC leachate treatment (Tukey-
243 HSD, $p_{\text{adj}}=0.02$). In contrast, genes belonging to the MLS category (macrolides,
244 lincosamides, and streptogramins) were significantly lower in abundance in both 1% and
245 10% PVC leachate treatments compared to the seawater control (Tukey-HSD, $p_{\text{adj}}=0.04$ and
246 $p_{\text{adj}}=0.005$ respectively) (Suppl. Table 3b). As MLS antibiotics are primarily active against
247 Gram positive bacteria, these resistance genes are typically found in these organisms. Thus,
248 this decline in MLS resistance gene abundance may be due to the large increase in relative
249 abundance of Gram negative bacteria following leachate exposure (Focardi et al., 2022).

250 The profiles of resistance mechanisms within PVC leachate-treated communities were
251 also altered (Fig. 3b). In particular, the 10% PVC leachate treatment led to a significant
252 enrichment of genes that confer AMR via antibiotic efflux (Tukey-HSD, $p=0.01$) and
253 antibiotic target alteration (Tukey-HSD, $p<0.01$) mechanisms compared to untreated seawater
254 (Suppl. Table 4b). These two resistance mechanism categories encompass the majority of
255 AMR genes identified in this study (46% assigned to antibiotic efflux, 21% assigned to
256 antibiotic target alteration). Resistance genes assigned to the antibiotic target protection
257 category were significantly lower in abundance in 10% PVC compared to seawater, however,
258 this is a small category with fewer than 4% of AMR genes assigned to it overall.

259 The twenty most enriched AMR genes, showing the highest fold change in the 10%
260 PVC leachate treatment, were examined to determine their likely host organism and which
261 AMR category and resistance mechanism each was assigned to (Table 1).

262 Twelve out of the twenty most enriched antibiotic resistance genes are related to
263 antibiotic efflux. These include efflux pumps from the RND, SMR and MATE multidrug
264 efflux pump families, as well as MexT and BaeR, which are regulators of RND efflux pump
265 gene expression (Henderson et al., 2021). All three of these efflux pump families typically
266 have broad substrate specificities, particularly the RND efflux pumps. In addition to

267 antibiotics, these efflux pumps can often export a wide range of complex hydrophobic
 268 organic molecules. Thus, it is possible that the increased abundance of efflux pumps
 269 following exposure to plastic leachate may be due to their ability to protect against toxic
 270 organic components in the leachate, exporting such components out of the cell. Of the
 271 remaining most abundant resistance genes, three are target site alteration and all of these are
 272 *ugd* genes, which provide polymyxin resistance via lipopolysaccharide modification, and the
 273 beta-lactamase gene, *ampC*, involved in antibiotic inactivation.

274 **Table 1.** Characteristics of the most highly enriched AMR genes following 10% PVC
 275 exposure.¹

AMR Gene	Gene ID	Fold Change PVC10:SW (log ₂)	Mean Relative Abundance in PVC10 (TPM)	AMR Category	Resistance Mechanism	Predicted genus
<i>ampC</i>	c_000000000144_14	∞	10.8 ± 7	beta-lactam	Antibiotic inactivation	<i>Tritonibacter</i>
<i>emrE</i>	c_000000000007_139	∞	9.6 ± 6.5	multidrug	Antibiotic efflux	<i>Tritonibacter</i>
<i>mtrE</i>	c_000000000080_44	∞	9.4 ± 5.3	multidrug	Antibiotic efflux	<i>Tritonibacter</i>
<i>ugd</i>	c_000000000038_92	8.8	14.4 ± 9.8	peptide	Antibiotic target alteration	<i>Tritonibacter</i>
<i>abeS</i>	c_000000000316_5	7.6	22.7 ± 17.4	multidrug	Antibiotic efflux	<i>Pseudoalteromonas</i>
<i>acrB</i>	c_000000000018_83	6.8	77.6 ± 45.9	multidrug	Antibiotic efflux	<i>Alteromonas</i>
<i>ugd</i>	c_000000000027_138	6.8	64.6 ± 39.3	peptide	Antibiotic target alteration	<i>Alteromonas</i>
<i>ksgA</i>	c_000000000010_130	6.8	69.3 ± 41.1	aminoglycoside	-	<i>Alteromonas</i>
<i>mexT</i>	c_000000000025_20	6.7	56.7 ± 34.5	multidrug	Antibiotic efflux	<i>Alteromonas</i>

<i>adeF</i>	c_000000000005_64	6.6	66.9 ± 39	multidrug	Antibiotic efflux	<i>Alteromonas</i>
<i>baeR</i>	c_0000000000073_7	6	60.8 ± 37.1	aminoglycoside: aminocoumarin	Antibiotic efflux	<i>Alteromonas</i>
<i>mexT</i>	c_000000001693_3	5.9	17.9 ± 14.5	multidrug	Antibiotic efflux	<i>Alteromonas</i>
<i>ksgA</i>	c_000000011168_2	5.3	16.3 ± 12.8	aminoglycoside	-	<i>Paraglaciecola</i>
<i>mexT</i>	c_000000009727_1	5.2	19.8 ± 13.8	multidrug	Antibiotic efflux	<i>Alteromonas</i>
<i>pmpM</i>	c_000000003002_4	4.1	10.7 ± 6.7	multidrug	Antibiotic efflux	<i>Alcanivorax</i>
<i>adeF</i>	c_000000085939_101	4	10.5 ± 6.4	multidrug	Antibiotic efflux	<i>Alcanivorax</i>
<i>qepA</i>	c_000000000289_9	3.8	13 ± 8	fluoroquinolone	-	<i>Alcanivorax</i>
<i>ugd</i>	c_000000006705_2	3.7	20.5 ± 15	peptide	Antibiotic target alteration	<i>Vibrio</i>
<i>crp</i>	c_000000000481_8	3.7	11.3 ± 6.9	unclassified	-	<i>Alcanivorax</i>
<i>baeR</i>	c_000000005224_3	3.7	13.4 ± 8.2	aminoglycoside: aminocoumarin	Antibiotic efflux	<i>Alcanivorax</i>

276 ¹The AMR gene, category, resistance mechanism (as provided by PathoFact), and predicted genus (based on
 277 NCBI BLASTn) for AMR genes which were found to be highly enriched in 10% PVC treatments, sorted by fold
 278 change (log₂). Fold change has been reported as ∞ for genes which were not observed in the seawater
 279 community.

280

281 The most highly enriched AMR genes are all predicted to be found in heterotrophic,
 282 predominantly Gram negative bacteria in the microcosms, with *Tritonibacter*, *Alteromonas*
 283 and *Alcanivorax* the most common predicted hosts of these genes. This is consistent with
 284 what taxonomic groups were observed to be most enriched in the PVC treated samples
 285 (Focardi et al., 2022). *Tritonibacter* are marine bacteria, originally described from a cultured
 286 representative isolated from oil-contaminated surface water during the Deepwater Horizon oil
 287 spill (Klotz et al., 2018). *Alteromonas* has been reported to be one of the main groups of
 288 microbes capable of growing in plastic leachates (Birnstiel et al., 2022). *Alcanivorax* are

289 alkane degrading marine bacteria that are found in low abundance in surface marine waters
290 but are highly enriched in oil contaminated marine environments (Hara et al., 2003) and have
291 previously been reported to encode multiple multidrug resistance proteins (Sinha et al.,
292 2021).

293 While none of the genera containing these abundant AMR genes include known
294 human pathogens, with the exception of *Vibrio*, there is potential for gene transfer events,
295 facilitated by mobile genetic elements, to move AMR genes between lineages, and into
296 species which may pose a risk to human health. At least in *Escherichia coli*, plastic leachate
297 has been shown to upregulate horizontal gene transfer (Yuan et al., 2022), opening the
298 possibility of synergistic effects that enrich bacteria harbouring AMR genes, whilst also
299 facilitating AMR spread. Indeed, capture of AMR genes by mobile genetic elements has been
300 well documented, and in many cases, has resulted in their spread into diverse human
301 pathogens across the globe, originating from single mobilisation events (Moellering Jr, 2010;
302 Wang et al., 2018). Further, a large proportion of AMR genes now globally circulating
303 among clinical pathogens are predicted to have originated in marine environments, including
304 several efflux pump and beta-lactamase genes (Ghaly et al., 2021). *Alteromonas* species
305 harbour large conjugative elements that can facilitate this movement, such as mega-plasmids
306 and integrative and conjugative elements (ICEs) (Cusick et al., 2020; López-Pérez et al.,
307 2017). In fact, several characterised ICEs are shared between *Alteromonas* and human
308 pathogens (López-Pérez et al., 2017; Pang et al., 2016), indicating the potential transmission
309 of genes from environmental to clinical organisms.

310

311 *3.3. Plastic leachate exposure changes the composition of virulence factors*

312

313 PVC leachate treatments drove clear shifts in virulence gene profiles (SeqScreen-derived),
314 evident from NMDS analysis (Fig. 4a, stress value = 0.05, indicating clear separation from
315 both the control and zinc treatments) and supported by PERMANOVA ($p=0.001$, $R^2 = 0.59$,
316 Suppl. Table 6a). PVC 10% treatment had a significant negative effect on the Shannon-
317 Wiener diversity of virulence genes (Fig. 4b; Tukey-HSD, p adj = 0.004, Suppl. Table 6c),
318 however, no such effect was observed on Simpson diversity (Fig 4c). This indicates that
319 enrichment of virulence genes following PVC leachate exposure is due to an increase in the
320 relative abundance of specific virulence genes, rather than an increase in overall virulence
321 gene diversity.

322 PVC leachate treatments led to changes in the composition of virulence genes, based
323 on SeqScreen assigned virulence categories (Fig. 5). Both 1% and 10% PVC treatments
324 resulted in significant increases in the relative abundance of two virulence categories:
325 secretion (Tukey-HSD, p -adj = 0.003 for PVC1, p -adj < 0.0001 for PVC10), and bacterial
326 counter signalling (Tukey-HSD, p -adj = 0.026 for PVC1, p -adj < 0.0001 for PVC10) (Suppl.
327 Table 7b). The secretion category includes the components of bacterial secretion systems, and
328 the bacterial counter signalling category includes genes involved in the suppression of host
329 immune signalling to avoid inflammatory responses. The toxin synthase category, however,
330 was significantly reduced in PVC10 samples (Tukey-HSD, p adj = 0.005, Suppl. Table 7b).
331 This category includes enzymes involved in the production or modification of toxins. In the
332 SeqScreen database, this category is largely focused on mycotoxins (those synthesised by
333 fungi). Plastic leachate has toxic effects on fungi, impairing fungal enzymatic activity (Li et
334 al., 2022). Thus, a decline in this category may be due to the negative effects of PVC leachate
335 exposure on marine fungi within the seawater microcosm.

336 Analysis of the virulence genes most strongly enriched in the 10% PVC leachate
337 treatment was carried out to determine their likely host organism and which virulence

338 category each falls under. Table 2 lists the twenty genes with the highest fold change
339 enrichment.

340 Sixteen out of the twenty most enriched genes are involved in secretion, encoding
341 components of the general secretion (Sec) pathway and Type II secretion systems (T2SSs).
342 The Sec pathway is used by several bacterial pathogens to secrete proteins that promote their
343 virulence (Green and Meccas, 2016). Although the Sec pathway does not export proteins
344 outside of the cell, in Gram negative bacteria, proteins delivered by the Sec pathway to the
345 periplasm can be exported with the aid of T2SSs (Green and Meccas, 2016). T2SS channels
346 are located only in the outer membrane, and thus can only export proteins that have been
347 delivered to the periplasm by other pathways, including the Sec pathway (Korotkov et al.,
348 2012). Thus, the simultaneous enrichment of both T2SS and Sec pathway components
349 suggests that PVC leachate exposure leads to an increase in microbes that employ
350 extracellular protein secretion. Several bacterial pathogens use T2SSs to secrete proteins
351 associated with host disease, such as hemolysins, lipases, proteases, esterases,
352 polygalacturonases, deubiquitinases, aerolysins, DNases, amylases, and mucin-degrading
353 enzymes (Cianciotto and White, 2017).

354 *Alteromonas* spp. appear to be largely responsible for driving the increase in virulence
355 genes following PVC leachate exposure (Table 2). Several *Alteromonas* spp. have been
356 reported as coral and algal pathogens (Brown et al., 2013; Peng and Li, 2013; Vairappan et
357 al., 2001), and associated with disease in marine arthropods (Alfiansah et al., 2020). Plastic
358 pollution has been shown to have toxic effects on both algae and marine invertebrates
359 (Haegerbaeumer et al., 2019; Pisani et al., 2022; Simon et al., 2021; Zhu et al., 2022), and
360 entanglement by plastic particles may significantly increase the risk of disease in
361 scleractinian corals (Lamb et al., 2018). Here, we show that an additional consequence of

362 plastic pollution for these organisms might be greater disease susceptibility due to the
 363 enrichment of pathogenic bacteria and their associated virulence traits.

364

365 **Table 2.** Top 20 Virulence genes enriched in 10% PVC treatments, sorted by fold change
 366 (\log_2) based on SeqScreen analyses.

Virulence Protein	Gene ID	Fold		Virulence Categories	Predicted Genus
		Change SW (\log_2)	Relative Abundance in PVC10 (TPM)		
GemA protein	c_000000002085_2	9.3	52.7 ± 51.4	Host cell cycle	<i>Muvirus</i> (Phage)
General secretion pathway protein H	c_000000000015_43	8	78.7 ± 46.9	Secretion	<i>Alteromonas</i>
PKS_ER domain-containing protein	c_000000000003_132	7.8	68.4 ± 40.4	Toxin synthase	<i>Pseudomonas</i>
Type II secretion system protein GspC	c_000000000015_38	7.4	80.6 ± 47.1	Secretion	<i>Alteromonas</i>
General secretion pathway protein H	c_000000000425_11	7.3	70.5 ± 41.6	Secretion	<i>Alteromonas</i>
Cyclic pyranopterin monophosphate synthase	c_000000000126_22	7.3	60 ± 37.6	Bacterial counter signalling	<i>Alteromonas</i>
Type II secretion system core protein G	c_000000000015_42	7.3	85.2 ± 50.1	Secretion	<i>Alteromonas</i>
Type II secretion system protein E	c_000000000015_40	7.2	77.6 ± 45.9	Secretion	<i>Alteromonas</i>
General secretion pathway protein H	c_000000000034_25	7	41.2 ± 26.7	Secretion	<i>Alteromonas</i>
General secretion pathway protein F	c_000000000005_71	6.9	62.7 ± 36.5	Secretion	<i>Alteromonas</i>

General secretion pathway protein E	c_000000000022_5	6.9	71.2 ± 42.4	Secretion	<i>Alteromonas</i>
General secretion pathway protein GspD	c_000000000015_39	6.9	81.3 ± 47.6	Secretion	<i>Alteromonas</i>
Type II secretion system protein J	c_000000000015_45	6.9	78.1 ± 44.3	Secretion	<i>Alteromonas</i>
Type II secretion system protein L	c_000000000015_47	6.9	80.7 ± 47.6	Secretion	<i>Alteromonas</i>
Sec-independent protein translocase protein TatA	c_000000000022_115	6.8	60.4 ± 35.7	Secretion	<i>Alteromonas</i>
General secretion pathway protein F	c_000000000015_41	6.7	79.1 ± 48	Secretion	<i>Alteromonas</i>
GspH domain-containing protein	c_000000000025_100	6.5	62.9 ± 37.9	Secretion	<i>Alteromonas</i>
Cyclic pyranopterin monophosphate synthase	c_000001149559_2	6.5	61.7 ± 35	Bacterial counter signalling	<i>Alteromonas</i>
GspH domain-containing protein	c_000000000025_102	6.5	63.9 ± 38	Secretion	<i>Alteromonas</i>
Type II secretion system protein GspD	c_000000000003_326	6.4	60.9 ± 36.8	Secretion	<i>Phycisphaerae</i> family

367

368

369 **4. Conclusion**

370

371 There is growing evidence that plastic pollution in marine environments can lead to an
372 enrichment in pathogenic bacteria and AMR genes. However, it is unclear whether these
373 effects are driven by the physical or chemical attributes of plastic marine pollution, as
374 differential colonisation and growth rates on plastic particles may be driven by physical
375 surface properties and/or chemicals leached from the plastic. Here we show that PVC
376 leachate, in the absence of plastic surfaces, drives an enrichment in AMR and virulence genes
377 within a seawater community. The enrichment of pathogenic bacteria and virulence traits

378 may have serious consequences for environments which are frequently exposed to human
379 pollution, such as urban harbours and aquacultural settings. Aquacultural systems are
380 especially vulnerable, as they are exposed to extreme levels of plastic pollution and provide
381 conditions ideal for disease emergence and spread.

382 From a One Health perspective, the selection for AMR genes in non-clinical settings
383 may pose a serious risk to human health. Although, the most strongly enriched AMR genes in
384 the present study were generally found in species not known to be human pathogens, there is
385 potential for horizontal transfer events to move these genes into species of clinical relevance.
386 Indeed, environmental bacteria can not only act as vectors for the transmission of AMR
387 genes, but also as their sources. Thus, the addition of selective forces that drive the
388 enrichment of AMR genes in environmental settings can contribute to their biogeographic
389 expansion. Such processes need only point sources of AMR genes to have global
390 consequences.

391 Given the widespread problem of plastic waste entering the environment, the
392 consequent enrichment of AMR and pathogenic traits is likely occurring in polluted sites
393 worldwide. Such changes pose an interconnected risk to plant, animal, and human health,
394 with the potential to further fuel the global resistance crisis and increase the total burden of
395 disease among marine macroorganisms.

396

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398

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401

402 **Author contributions**

403

404 ST and EV designed the study. EV, AF, and TG performed the data analyses. All authors
405 contributed to data interpretation, writing the original draft, and reviewed and edited the final
406 draft.

407

408 **References**

409

- 410 Alfiansah, Y.R., Peters, S., Harder, J., Hassenrück, C. and Gärdes, A. 2020. Structure and co-
411 occurrence patterns of bacterial communities associated with white faeces disease
412 outbreaks in Pacific white-leg shrimp *Penaeus vannamei* aquaculture. Scientific
413 Reports 10(1), 11980.
- 414 Balaji, A., Kille, B., Kappell, A.D., Godbold, G.D., Diep, M., Elworth, R.A.L., Qian, Z., Albin, D.,
415 Nasko, D.J., Shah, N., Pop, M., Segarra, S., Ternus, K.L. and Treangen, T.J. 2022.
416 SeqScreen: accurate and sensitive functional screening of pathogenic sequences via
417 ensemble learning. Genome Biology 23(1), 133.
- 418 Bhagwat, G., Zhu, Q., O'Connor, W., Subashchandrabose, S., Grainge, I., Knight, R. and
419 Palanisami, T. 2021. Exploring the Composition and Functions of Plastic Microbiome
420 Using Whole-Genome Sequencing. Environmental Science & Technology 55(8), 4899-
421 4913.
- 422 Birnstiel, S., Sebastián, M. and Romera-Castillo, C. 2022. Structure and activity of marine
423 bacterial communities responding to plastic leachates. Science of The Total
424 Environment 834, 155264.
- 425 Borrelle, S.B., Ringma, J., Law, K.L., Monnahan, C.C., Lebreton, L., McGivern, A., Murphy, E.,
426 Jambeck, J., Leonard, G.H., Hilleary, M.A., Eriksen, M., Possingham, H.P., De Frond,
427 H., Gerber, L.R., Polidoro, B., Tahir, A., Bernard, M., Mallos, N., Barnes, M. and
428 Rochman, C.M. 2020. Predicted growth in plastic waste exceeds efforts to mitigate
429 plastic pollution. Science 369(6510), 1515-1518.
- 430 Brown, T., Bourne, D. and Rodriguez-Lanetty, M. 2013. Transcriptional activation of *c3* and
431 *hsp70* as part of the immune response of *Acropora millepora* to bacterial challenges.
432 PLoS One 8(7), e67246.
- 433 Bryant, J., A., Clemente, T., M., Viviani, D., A., Fong, A., A., Thomas, K., A., Kemp, P., Karl, D.,
434 M., White, A., E., DeLong, E., F. and Jansson, J., K. 2016. Diversity and Activity of
435 Communities Inhabiting Plastic Debris in the North Pacific Gyre. mSystems 1(3),
436 e00024-00016.
- 437 Buchfink, B., Xie, C. and Huson, D.H. 2015. Fast and sensitive protein alignment using
438 DIAMOND. Nature Methods 12(1), 59-60.
- 439 Capolupo, M., Sørensen, L., Jayasena, K.D.R., Booth, A.M. and Fabbri, E. 2020. Chemical
440 composition and ecotoxicity of plastic and car tire rubber leachates to aquatic
441 organisms. Water Research 169, 115270.
- 442 Cianciotto, N.P. and White, R.C. 2017. Expanding role of type II secretion in bacterial
443 pathogenesis and beyond. Infection and Immunity 85(5), e00014-00017.

- 444 Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S.,
445 Palma, Á.T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernández-de-Puelles, M.L. and
446 Duarte, C.M. 2014. Plastic debris in the open ocean. *Proceedings of the National
447 Academy of Sciences* 111(28), 10239-10244.
- 448 Cusick, K.D., Polson, S.W., Duran, G. and Hill, R.T. 2020. Multiple megaplasmids confer
449 extremely high levels of metal tolerance in *Alteromonas* strains. *Applied and
450 Environmental Microbiology* 86(3), e01831-01819.
- 451 de Nies, L., Lopes, S., Busi, S.B., Galata, V., Heintz-Buschart, A., Laczny, C.C., May, P. and
452 Wilmes, P. 2021. PathoFact: a pipeline for the prediction of virulence factors and
453 antimicrobial resistance genes in metagenomic data. *Microbiome* 9(1), 49.
- 454 Di Pippo, F., Crognale, S., Levantesi, C., Vitanza, L., Sighicelli, M., Pietrelli, L., Di Vito, S.,
455 Amalfitano, S. and Rossetti, S. 2022. Plasticsphere in lake waters: Microbial diversity,
456 biofilm structure, and potential implications for freshwater ecosystems.
457 *Environmental Pollution* 310, 119876.
- 458 Dussud, C., Meistertzheim, A.L., Conan, P., Pujó-Pay, M., George, M., Fabre, P., Coudane, J.,
459 Higgs, P., Elineau, A., Pedrotti, M.L., Gorsky, G. and Ghiglione, J.F. 2018. Evidence of
460 niche partitioning among bacteria living on plastics, organic particles and
461 surrounding seawaters. *Environmental Pollution* 236, 807-816.
- 462 Focardi, A., Moore, L.R., Raina, J.B., Seymour, J.R., Paulsen, I.T. and Tetu, S.G. 2022. Plastic
463 leachates impair picophytoplankton and dramatically reshape the marine
464 microbiome. *Microbiome* 10, 179.
- 465 Gewert, B., MacLeod, M. and Breitholtz, M. 2021. Variability in Toxicity of Plastic Leachates
466 as a Function of Weathering and Polymer Type: A Screening Study with the Copepod
467 *Nitocra spinipes*. *The Biological Bulletin* 240(3), 191-199.
- 468 Ghaly, T.M., Tetu, S.G. and Gillings, M.R. 2021. Predicting the taxonomic and
469 environmental sources of integron gene cassettes using structural and sequence
470 homology of *attC* sites. *Communications Biology* 4(1), 946.
- 471 Green, E.R. and Meccas, J. 2016. Bacterial secretion systems: an overview. *Microbiology
472 Spectrum* 4(1), 4.1.13.
- 473 Gregory, M.R. 2009. Environmental implications of plastic debris in marine settings—
474 entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions.
475 *Phil. Trans. R. Soc. B* 364, 2013 - 2025.
- 476 Gunaalan, K., Fabbri, E. and Capolupo, M. 2020. The hidden threat of plastic leachates: A
477 critical review on their impacts on aquatic organisms. *Water Research* 184, 116170.
- 478 Haegerbaeumer, A., Mueller, M.-T., Fueser, H. and Traunspurger, W. 2019. Impacts of
479 micro- and nano-sized plastic particles on benthic invertebrates: a literature review
480 and gap analysis. *Frontiers in Environmental Science* 7, 17.
- 481 Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E. and Purnell, P. 2018. An overview of
482 chemical additives present in plastics: Migration, release, fate and environmental
483 impact during their use, disposal and recycling. *Journal of Hazardous Materials* 344,
484 179-199.
- 485 Hara, A., Syutsubo, K. and Harayama, S. 2003. *Alcanivorax* which prevails in
486 oil-contaminated seawater exhibits broad substrate specificity for alkane
487 degradation. *Environmental Microbiology* 5(9), 746-753.
- 488 He, S., Jia, M., Xiang, Y., Song, B., Xiong, W., Cao, J., Peng, H., Yang, Y., Wang, W., Yang, Z.
489 and Zeng, G. 2022. Biofilm on microplastics in aqueous environment:

- 490 physicochemical properties and environmental implications. *Journal of Hazardous*
491 *Materials* 424, 127286.
- 492 Henderson, P.J.F., Maher, C., Elbourne, L.D.H., Eijkelkamp, B.A., Paulsen, I.T. and Hassan,
493 K.A. 2021. Physiological functions of bacterial “multidrug” efflux pumps. *Chemical*
494 *Reviews* 121(9), 5417-5478.
- 495 Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P. and Duflos,
496 G. 2017. Occurrence and effects of plastic additives on marine environments and
497 organisms: A review. *Chemosphere* 182, 781-793.
- 498 Jadhav, H.S., Fulke, A.B. and Giripunje, M.D. 2022. Recent global insight into mitigation of
499 plastic pollutants, sustainable biodegradable alternatives, and recycling strategies.
500 *International Journal of Environmental Science and Technology*.
- 501 Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R. and
502 Law, K.L. 2015. Plastic waste inputs from land into the ocean. *Science* 347(6223),
503 768-771.
- 504 Kim, D., Song, L., Breitwieser, F.P. and Salzberg, S.L. 2016. Centrifuge: rapid and sensitive
505 classification of metagenomic sequences. *Genome Research* 26(12), 1721-1729.
- 506 Klotz, F., Brinkhoff, T., Freese, H.M., Wietz, M., Teske, A., Simon, M. and Giebel, H.-A. 2018.
507 *Tritonibacter horizontalis* gen. nov., sp. nov., a member of the Rhodobacteraceae,
508 isolated from the Deepwater Horizon oil spill. *International Journal of Systematic and*
509 *Evolutionary Microbiology* 68(3), 736-744.
- 510 Korotkov, K.V., Sandkvist, M. and Hol, W.G.J. 2012. The type II secretion system:
511 biogenesis, molecular architecture and mechanism. *Nature Reviews Microbiology*
512 10(5), 336-351.
- 513 Lamb, J.B., Willis, B.L., Fiorenza, E.A., Couch, C.S., Howard, R., Rader, D.N., True, J.D., Kelly,
514 L.A., Ahmad, A., Jompa, J. and Harvell, C.D. 2018. Plastic waste associated with
515 disease on coral reefs. *Science* 359(6374), 460-462.
- 516 Lau, W.W.Y., Shiran, Y., Bailey, R.M., Cook, E., Stuchtey, M.R., Koskella, J., Velis, C.A.,
517 Godfrey, L., Boucher, J., Murphy, M.B., Thompson, R.C., Jankowska, E., Castillo
518 Castillo, A., Pilditch, T.D., Dixon, B., Koerselman, L., Kosior, E., Favoino, E., Gutberlet,
519 J., Baulch, S., Atreya, M.E., Fischer, D., He, K.K., Petit, M.M., Sumaila, U.R., Neil, E.,
520 Bernhofen, M.V., Lawrence, K. and Palardy, J.E. 2020. Evaluating scenarios toward
521 zero plastic pollution. *Science* 369(6510), 1455-1461.
- 522 Lebreton, L. and Andrady, A. 2019. Future scenarios of global plastic waste generation and
523 disposal. *Palgrave Communications* 5(1), 6.
- 524 Lebreton, L., Slat, B., Ferrari, F., Sainte-Rose, B., Aitken, J., Marthouse, R., Hajbane, S.,
525 Cunsolo, S., Schwarz, A., Levivier, A., Noble, K., Debeljak, P., Maral, H., Schoeneich-
526 Argent, R., Brambini, R. and Reisser, J. 2018. Evidence that the Great Pacific
527 Garbage Patch is rapidly accumulating plastic. *Scientific Reports* 8(1), 4666.
- 528 Li, Z., Xie, Y., Zeng, Y., Zhang, Z., Song, Y., Hong, Z., Ma, L., He, M., Ma, H. and Cui, F. 2022.
529 Plastic leachates lead to long-term toxicity in fungi and promote biodegradation of
530 heterocyclic dye. *Science of The Total Environment* 806, 150538.
- 531 Lithner, D., Damberg, J., Dave, G. and Larsson, Å. 2009. Leachates from plastic consumer
532 products – Screening for toxicity with *Daphnia magna*. *Chemosphere* 74(9), 1195-
533 1200.
- 534 Loiseau, C. and Sorci, G. 2022. Can microplastics facilitate the emergence of infectious
535 diseases? *Science of the Total Environment* 823.

- 536 López-Pérez, M., Ramon-Marco, N. and Rodriguez-Valera, F. 2017. Networking in microbes:
537 conjugative elements and plasmids in the genus *Alteromonas*. BMC Genomics 18(1),
538 36.
- 539 Moellering Jr, R.C. 2010. NDM-1—a cause for worldwide concern. New England Journal of
540 Medicine 363(25), 2377-2379.
- 541 Oberbeckmann, S., Löder, M.G.J. and Labrenz, M. 2015. Marine microplastic-associated
542 biofilms – a review. Environmental Chemistry 12(5), 551-562.
- 543 Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O’Hara, R.B., Simpson, G.L.,
544 Solymos, P., Stevens, M.H.H., Wagner, H. and Oksanen, M. 2013. vegan: Community
545 ecology package. <https://CRAN.R-project.org/package=vegan>.
- 546 Pang, B., Du, P., Zhou, Z., Diao, B., Cui, Z., Zhou, H. and Kan, B. 2016. The transmission and
547 antibiotic resistance variation in a multiple drug resistance clade of *Vibrio cholerae*
548 circulating in multiple countries in Asia. PLoS One 11(3), e0149742.
- 549 Peng, Y. and Li, W. 2013. A bacterial pathogen infecting gametophytes of *Saccharina*
550 *japonica* (Laminariales, Phaeophyceae). Chinese Journal of Oceanology and
551 Limnology 31(2), 366-373.
- 552 Pisani, X.G., Lompré, J.S., Pires, A. and Greco, L.L. 2022. Plastics in scene: A review of the
553 effect of plastics in aquatic crustaceans. Environmental Research 212, 113484.
- 554 Poole, K. 2017. At the nexus of antibiotics and metals: the impact of Cu and Zn on antibiotic
555 activity and resistance. Trends in Microbiology 25(10), 820-832.
- 556 Radisic, V., Nimje, P.S., Bienfait, A.M. and Marathe, N.P. 2020. Marine Plastics from
557 Norwegian West Coast Carry Potentially Virulent Fish Pathogens and Opportunistic
558 Human Pathogens Harboring New Variants of Antibiotic Resistance Genes.
559 Microorganisms 8(8).
- 560 Romera-Castillo, C., Pinto, M., Langer, T.M., Álvarez-Salgado, X.A. and Herndl, G.J. 2018.
561 Dissolved organic carbon leaching from plastics stimulates microbial activity in the
562 ocean. Nature Communications 9(1), 1430.
- 563 Sarker, I., Moore, L.R., Paulsen, I.T. and Tetu, S.G. 2020. Assessing the toxicity of leachates
564 from weathered plastics on photosynthetic marine bacteria *Prochlorococcus*.
565 Frontiers in Marine Science 7, 571929.
- 566 Sathicq, M.B., Sabatino, R., Corno, G. and Di Cesare, A. 2021. Are microplastic particles a
567 hotspot for the spread and the persistence of antibiotic resistance in aquatic
568 systems? Environmental Pollution 279, 116896.
- 569 Silva, I., Tacão, M. and Henriques, I. 2021. Selection of antibiotic resistance by metals in a
570 riverine bacterial community. Chemosphere 263, 127936.
- 571 Simon, M., Hartmann, N.B. and Vollertsen, J. 2021. Accelerated Weathering Increases the
572 Release of Toxic Leachates from Microplastic Particles as Demonstrated through
573 Altered Toxicity to the Green Algae *Raphidocelis subcapitata*. Toxics 9(8).
- 574 Sinha, R.K., Krishnan, K.P. and Kurian, P.J. 2021. Complete genome sequence and
575 comparative genome analysis of *Alcanivorax* sp. IO_7, a marine alkane-degrading
576 bacterium isolated from hydrothermally-influenced deep seawater of southwest
577 Indian ridge. Genomics 113, 884-891.
- 578 Sucato, A., Vecchioni, L., Savoca, D., Presentato, A., Arculeo, M. and Alduina, R. 2021. A
579 comparative analysis of aquatic and polyethylene-associated antibiotic-resistant
580 microbiota in the Mediterranean Sea. Biology 10(3), 200.

- 581 Sun, Y., Cao, N., Duan, C., Wang, Q., Ding, C. and Wang, J. 2021. Selection of antibiotic
582 resistance genes on biodegradable and non-biodegradable microplastics. *Journal of*
583 *Hazardous Materials* 409.
- 584 Suzek, B.E., Huang, H., McGarvey, P., Mazumder, R. and Wu, C.H. 2007. UniRef:
585 comprehensive and non-redundant UniProt reference clusters. *Bioinformatics*
586 23(10), 1282-1288.
- 587 Tetu, S.G., Sarker, I., Schrameyer, V., Pickford, R., Elbourne, L.D.H., Moore, L.R. and Paulsen,
588 I.T. 2019. Plastic leachates impair growth and oxygen production in
589 *Prochlorococcus*, the ocean's most abundant photosynthetic bacteria.
590 *Communications Biology* 2(1), 184.
- 591 Vairappan, C.S., Suzuki, M., Motomura, T. and Ichimura, T. 2001. Pathogenic bacteria
592 associated with lesions and thallus bleaching symptoms in the Japanese kelp
593 *Laminaria religiosa* Miyabe (Laminariales, Phaeophyceae). *Hydrobiologia* 445(1),
594 183-191.
- 595 Wagner, G.P., Kin, K. and Lynch, V.J. 2012. Measurement of mRNA abundance using RNA-
596 seq data: RPKM measure is inconsistent among samples. *Theory in Biosciences*
597 131(4), 281-285.
- 598 Wang, R., van Dorp, L., Shaw, L.P., Bradley, P., Wang, Q., Wang, X., Jin, L., Zhang, Q., Liu, Y.,
599 Rieux, A., Dorai-Schneiders, T., Weinert, L.A., Iqbal, Z., Didelot, X., Wang, H. and
600 Balloux, F. 2018. The global distribution and spread of the mobilized colistin
601 resistance gene *mcr-1*. *Nature Communications* 9(1), 1179.
- 602 Wang, S., Xue, N., Li, W., Zhang, D., Pan, X. and Luo, Y. 2020. Selectively enrichment of
603 antibiotics and ARGs by microplastics in river, estuary and marine waters. *Science of*
604 *The Total Environment* 708, 134594.
- 605 Wiesinger, H., Wang, Z. and Hellweg, S. 2021. Deep Dive into Plastic Monomers, Additives,
606 and Processing Aids. *Environmental Science & Technology* 55(13), 9339-9351.
- 607 Wright, S.L., Thompson, R.C. and Galloway, T.S. 2013. The physical impacts of microplastics
608 on marine organisms: A review. *Environmental Pollution* 178, 483-492.
- 609 Wu, T., Gagnon, A., McGourty, K., DosSantos, R., Chanetsa, L., Zhang, B., Bello, D. and
610 Kelleher, S.L. 2021. Zinc exposure promotes commensal-to-pathogen transition in
611 *Pseudomonas aeruginosa* leading to mucosal inflammation and illness in mice.
612 *International Journal of Molecular Sciences* 22(24), 13321.
- 613 Yang, Y., Liu, G., Song, W., Ye, C., Lin, H., Li, Z. and Liu, W. 2019. Plastics in the marine
614 environment are reservoirs for antibiotic and metal resistance genes. *Environment*
615 *International* 123, 79-86.
- 616 Yuan, Q., Sun, R., Yu, P., Cheng, Y., Wu, W., Bao, J. and Alvarez, P.J.J. 2022. UV-aging of
617 microplastics increases proximal ARG donor-recipient adsorption and leaching of
618 chemicals that synergistically enhance antibiotic resistance propagation. *Journal of*
619 *Hazardous Materials* 427, 127895.
- 620 Zettler, E.R., Mincer, T.J. and Amaral-Zettler, L.A. 2013. Life in the "Plastisphere": Microbial
621 Communities on Plastic Marine Debris. *Environmental Science & Technology* 47(13),
622 7137-7146.
- 623 Zhang, E., Kim, M., Rueda, L., Rochman, C., VanWormer, E., Moore, J. and Shapiro, K. 2022.
624 Association of zoonotic protozoan parasites with microplastics in seawater and
625 implications for human and wildlife health. *Scientific Reports* 12(1), 6532.

- 626 Zhu, X., Teng, J., Xu, E.G., Zhao, J., Shan, E., Sun, C. and Wang, Q. 2022. Toxicokinetics and
627 toxicodynamics of plastic and metallic nanoparticles: A comparative study in shrimp.
628 Environmental Pollution 312, 120069.
- 629 Zimmermann, L., Dierkes, G., Ternes, T.A., Völker, C. and Wagner, M. 2019. Benchmarking
630 the in Vitro Toxicity and Chemical Composition of Plastic Consumer Products.
631 Environmental Science & Technology 53(19), 11467-11477.
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634 **Figure Captions**

635

636 **Fig. 1.** Relative abundance (TPM sum) of genes encoding; (a, d) AMR, (b, e) virulence, and
637 (c, f) toxin functions, predicted by PathoFact for 1% PVC leachate (PVC1), 10% PVC
638 leachate (PVC10), 0.13 mg/L zinc chloride (ZnL), and 1.3 mg/mL zinc chloride (ZnH)
639 treatments, compared with seawater (SW). Tukey-HSD adjusted p-values have been reported
640 for treatments which differ significantly from the control. The full set of statistical results for
641 these tests are provided in Supplementary Table 1(b-m).

642

643 **Fig. 2.** a) NMDS plot of AMR gene profiles for all samples, b) Shannon-Wiener, and c)
644 Simpson diversity of AMR genes for seawater controls (SW), and 1% PVC leachate (PVC1),
645 and 10% PVC leachate (PVC10) treatments.

646

647 **Fig. 3.** Comparison of the mean relative abundance (TPM sum) between PVC and seawater
648 samples for a) antimicrobial resistance categories and b) antibiotic resistance mechanisms.
649 Error bars indicate the standard error of the mean and stars (*) denote a significant difference
650 from the control (SW) (Tukey-HSD, $p < 0.05$). Full statistical results for tests displayed here
651 are provided in Supplementary Tables 3 and 4.

652

653 **Fig. 4.** a) NMDS plot of virulence gene profiles from SeqScreen for all samples, b) Shannon-
654 Wiener, and c) Simpson diversity of virulence genes for seawater controls (SW), and 1%
655 PVC leachate (PVC1), and 10% PVC leachate (PVC10) treatments.

656

657 **Fig. 5.** Comparison of the mean relative abundance (TPM sum) between PVC and seawater
658 samples for virulence categories (provided by SeqScreen). Error bars indicate the standard

659 error of the mean and stars (*) denote a significant difference from the control (SW) (Tukey-

660 HSD, $p_{\text{adj}} < 0.05$). Full statistical results for tests displayed here are provided in

661 Supplementary Table 7.

662

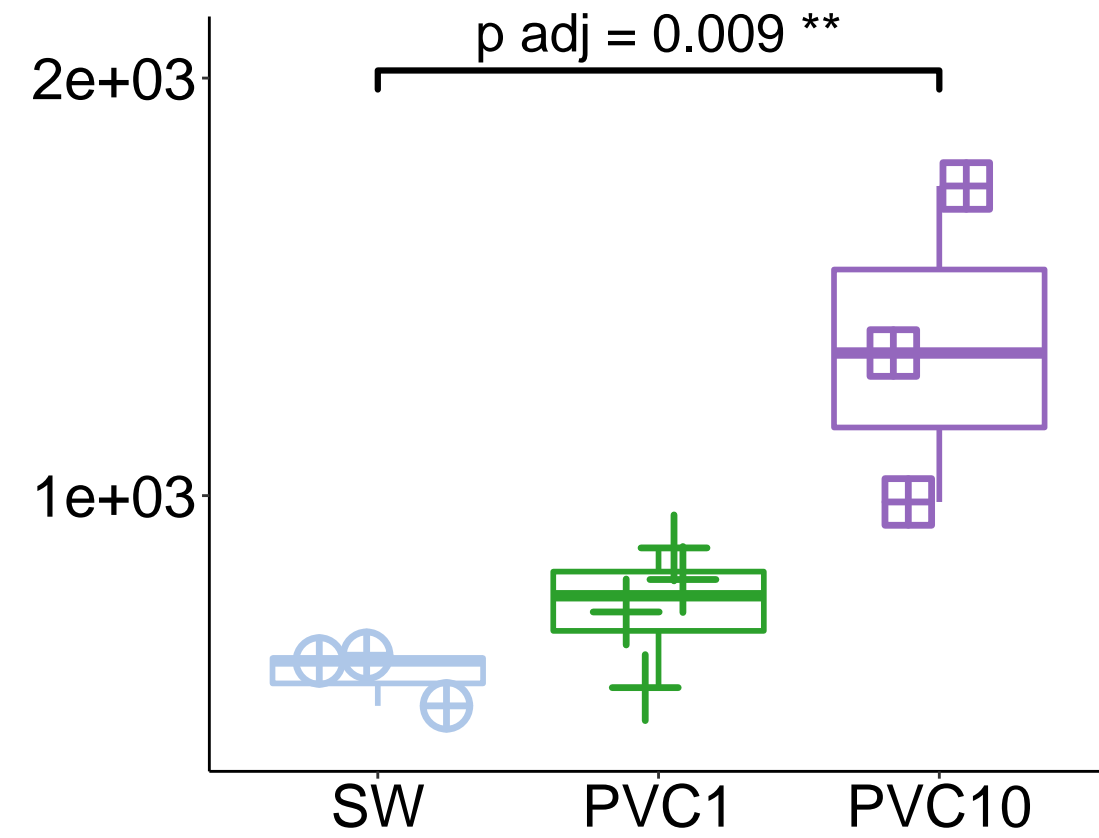
AMR

Virulence

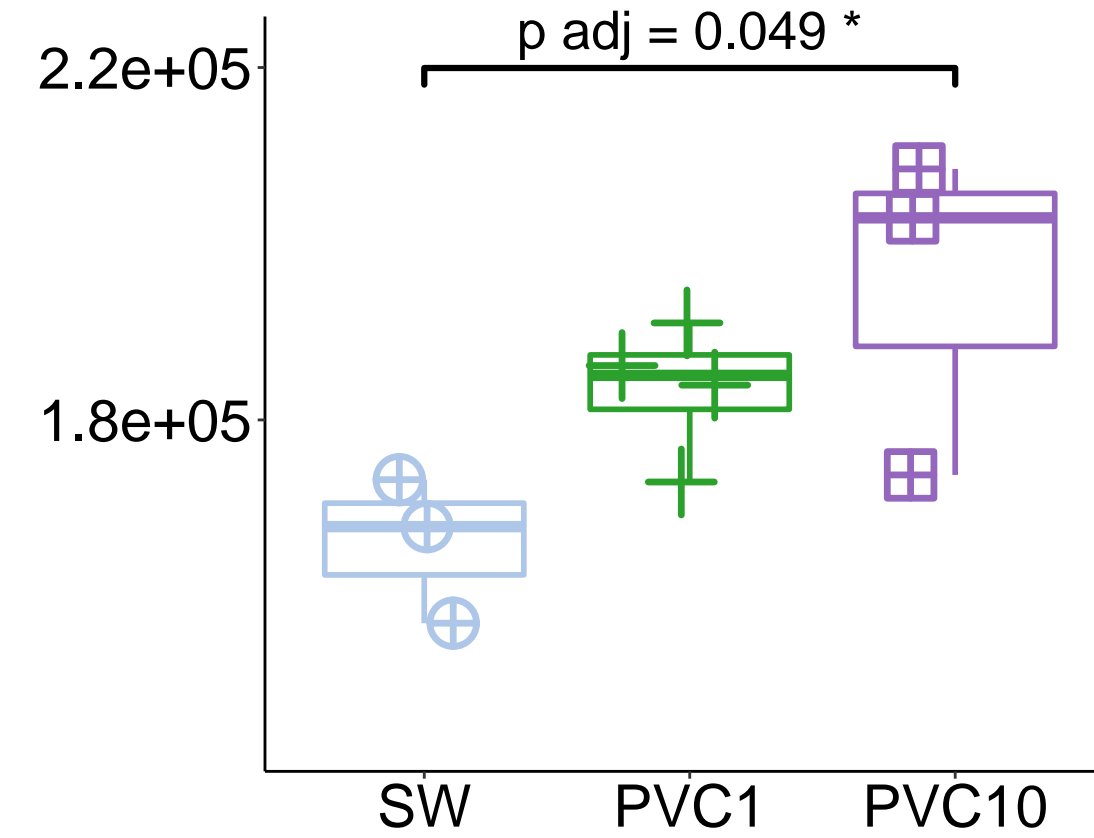
Toxin

a)

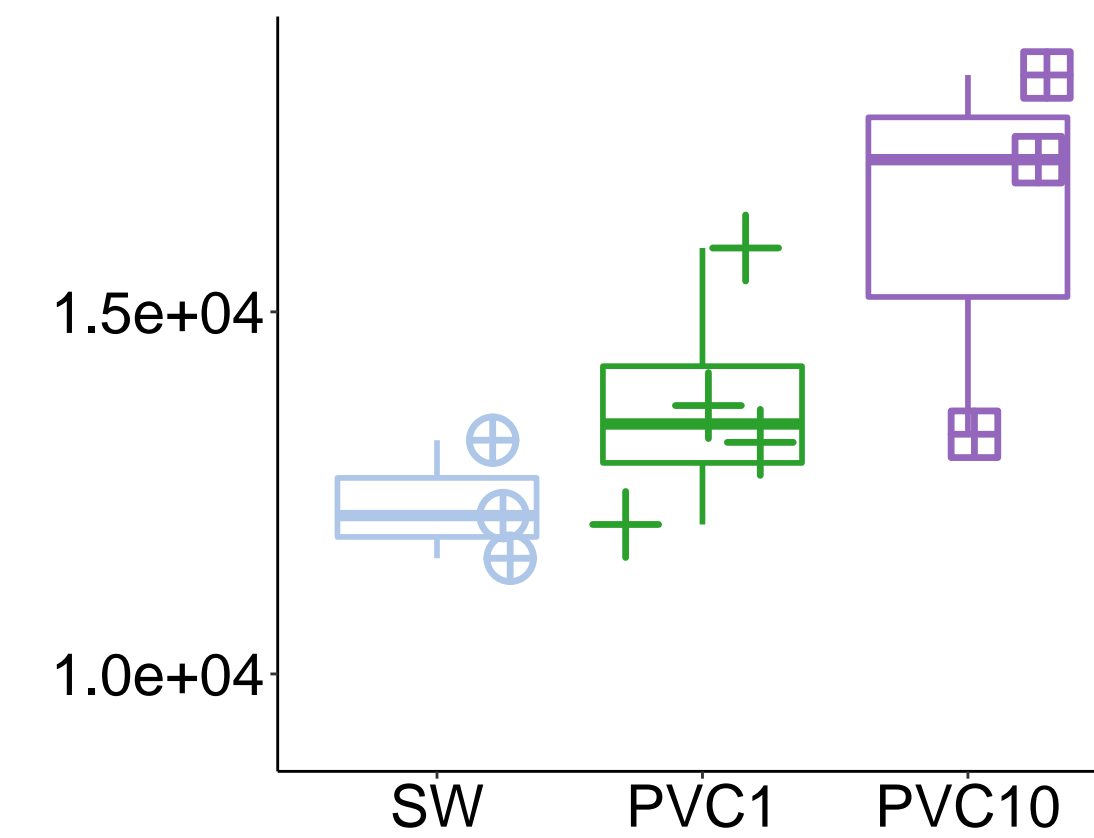
bioRxiv preprint doi: <https://doi.org/10.1101/2023.02.13.528379>; this version posted February 14, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.



b)



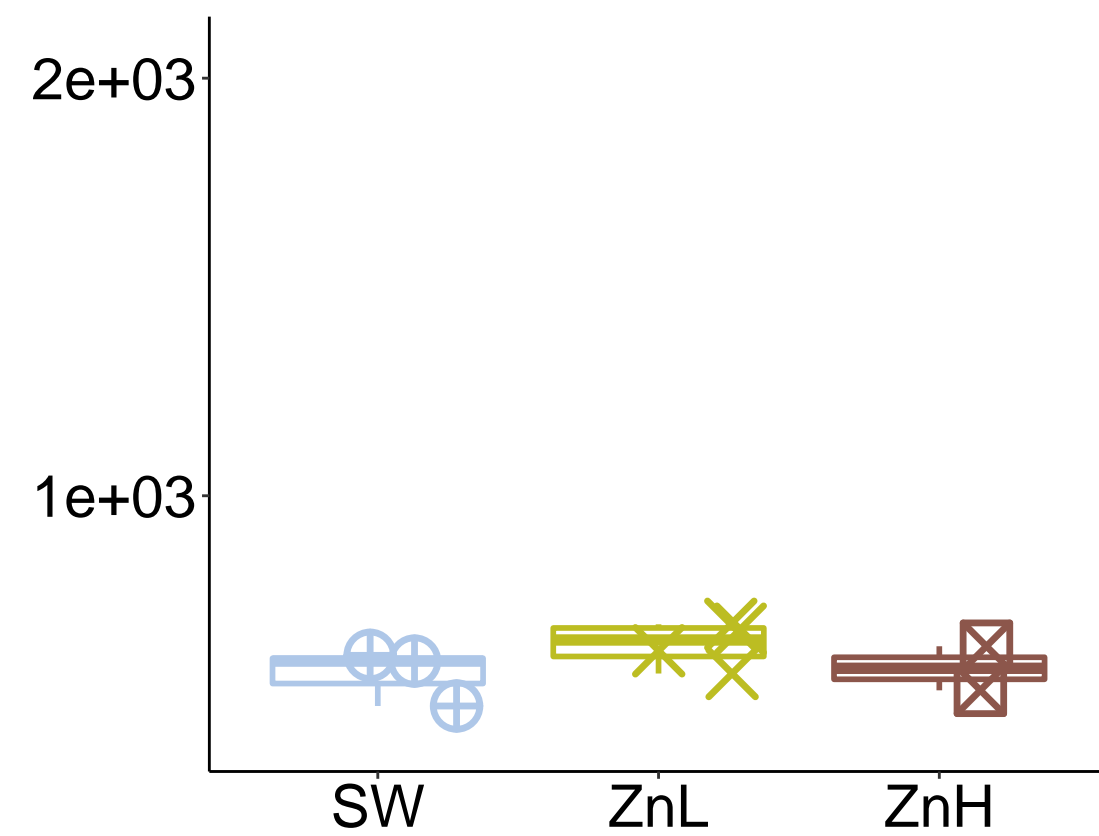
c)



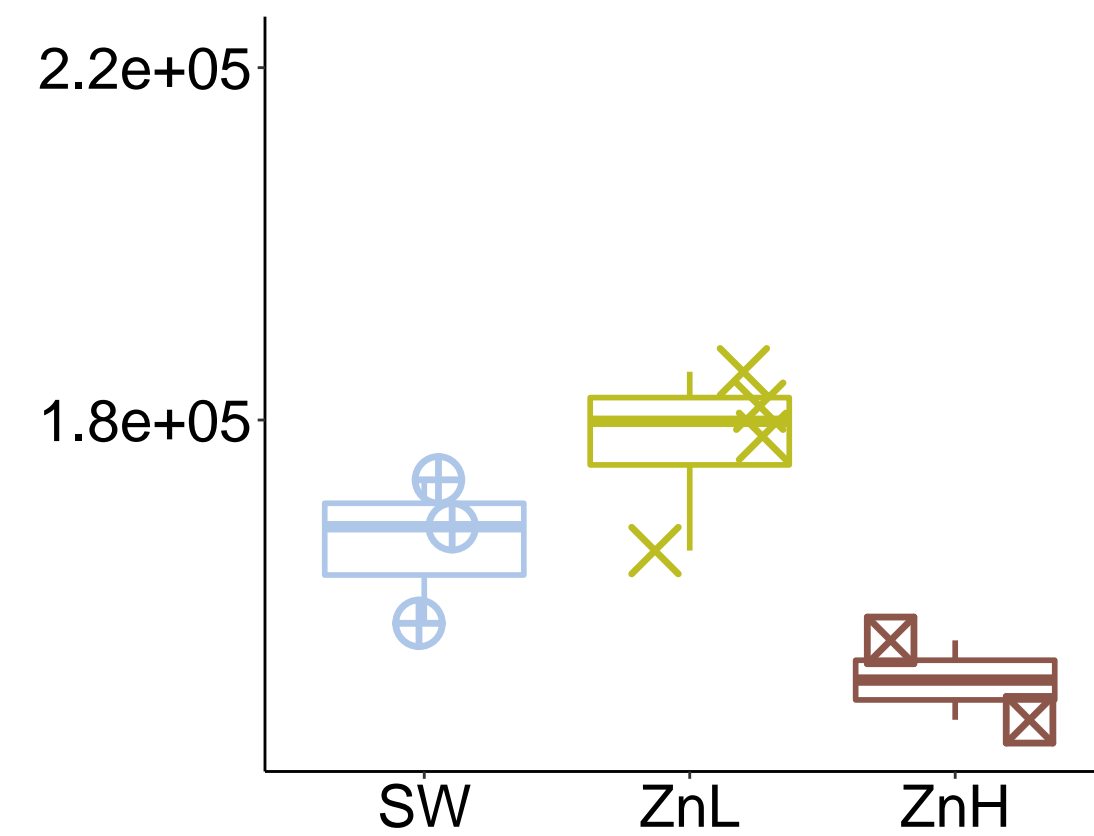
Treatment

- ⊕ SW
- + PVC1
- ⊞ PVC10
- × ZnL
- ⊠ ZnH

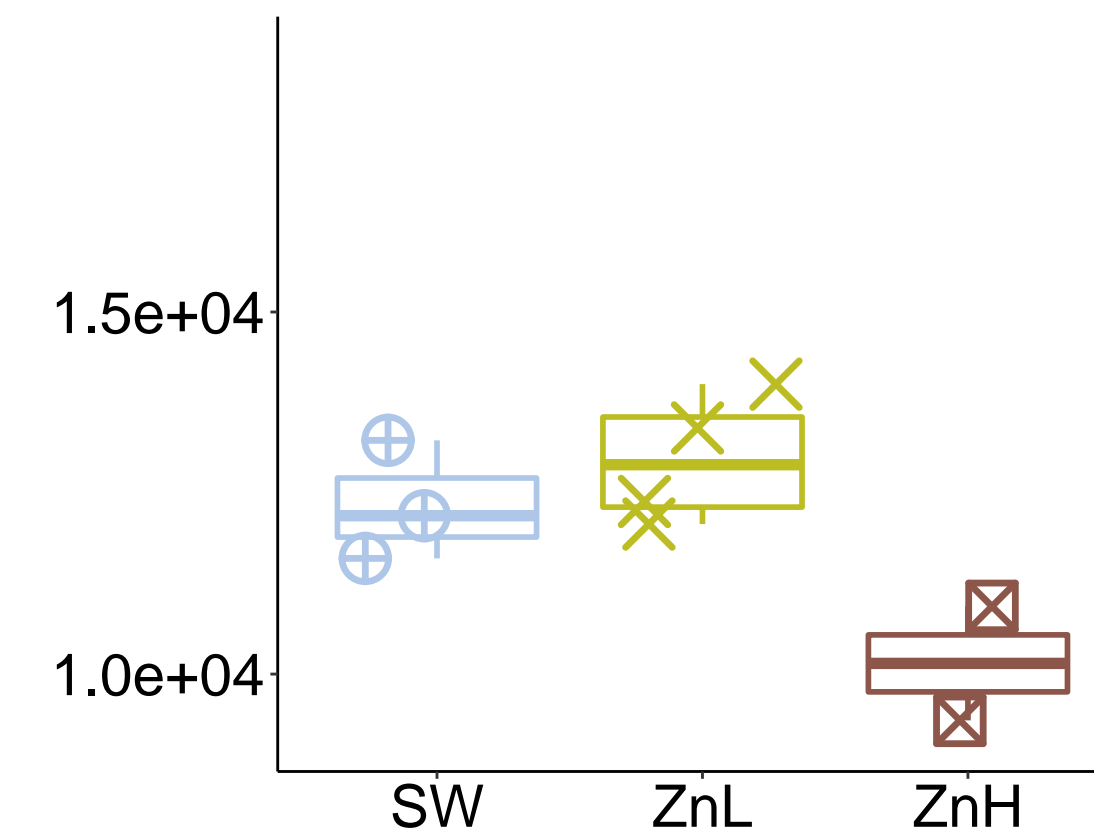
d)



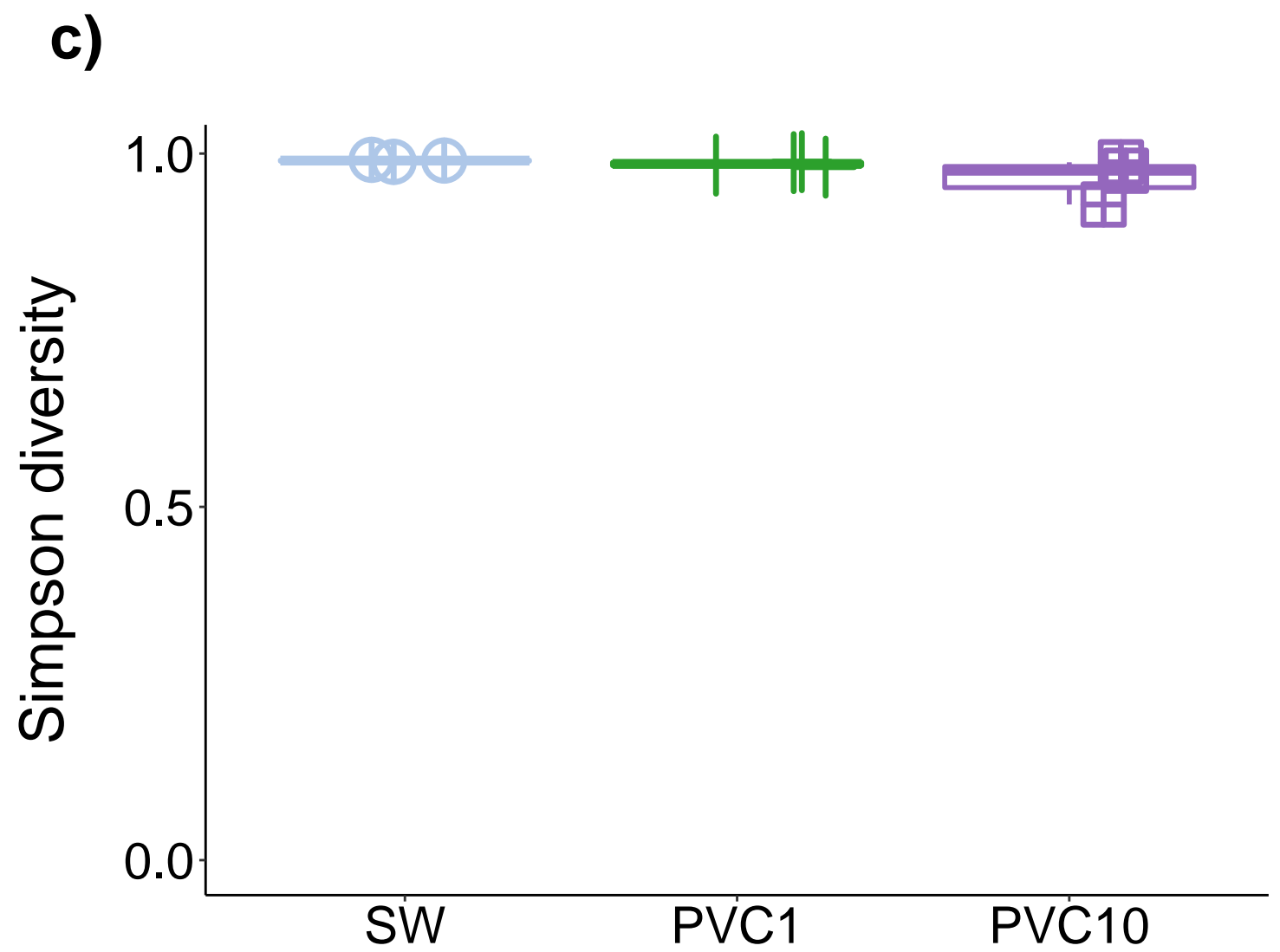
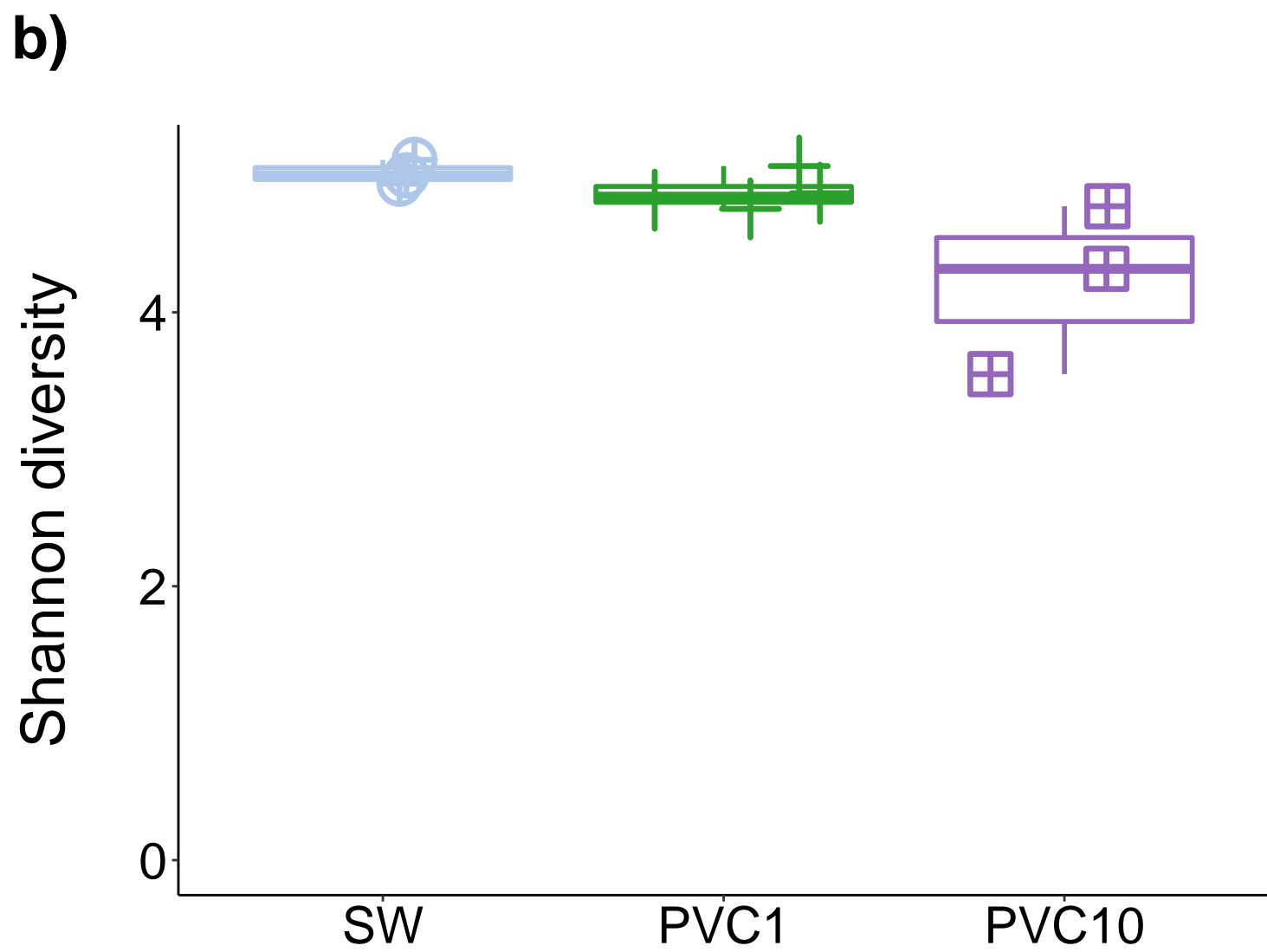
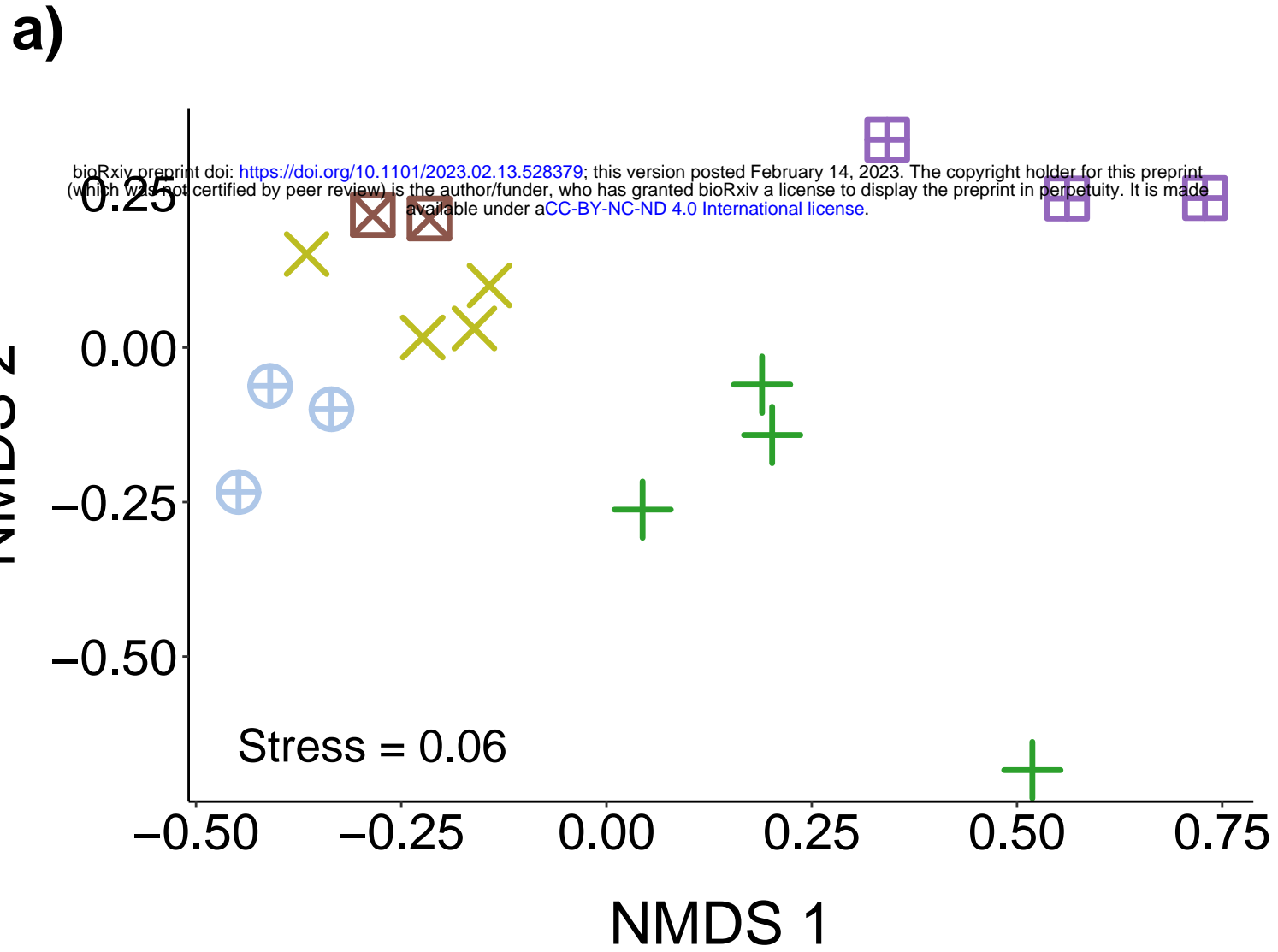
e)



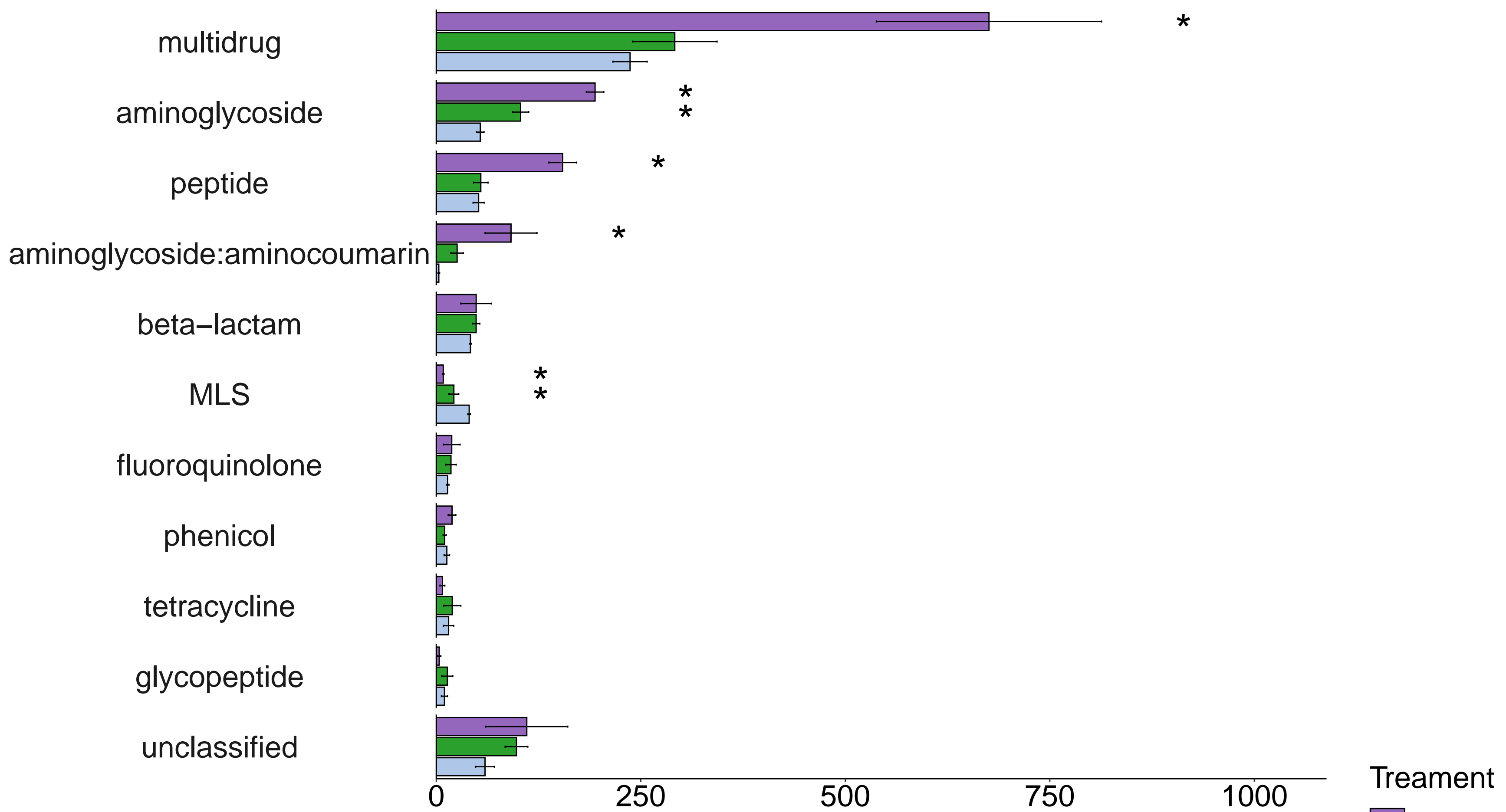
f)



Treatment



a) AMR categories



b) Resistance mechanisms

