Effects of microplastic ingestion on hydrogen production and microbiomes in the gut of the terrestrial isopod *Porcellio scaber*

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1 **Abstract**

2 Microplastic (MP) pollution is an environmental burden. MP enters food webs via ingestion by

3 macrofauna, including isopods (*Porcellio scaber*) in terrestrial ecosystems. However, MP-

4 effects on the host and its gut microbiome are largely unknown. We tested the hypothesis that

5 biodegradable (polylactic acid, PLA) and non-biodegradable (polyethylene terephthalate, PET;

6 polystyrene, PS) MP have contrasting effects on *P. scaber* mediated by changes of the

7 associated gut microbiome. Although the isopods avoided food containing PS, isopod fitness

8 after eight-week MP-exposure was unaffected. Qualitative and quantitative 16S rRNA gene

9 and 16S rRNA analyses of gut microbiomes indicated general MP effects, MP-type specific

10 indicator taxa, and stimulation by PLA compared to MP-free controls. Isopods emitted

11 hydrogen, and its production increased and decreased after PLA-food and PET- or PS-food

12 ingestion, respectively, relative to controls as indicated by microsensor measurements. Gut

13 pH was unaffected by MP. We identified the gut of *P. scaber* as significant mobile source of

14 reductant for soil microbiomes likely due to *Enterobacteriaceae* related fermentation activities

15 that were stimulated by lactate generated during PLA-degradation. The findings suggest

16 negative effects of PET and PS on gut fermentation, modulation of isopod hydrogen emissions

17 by MP pollution, and the potential of MP to affect terrestrial food webs.

18 **Introduction**

19 Today's modern life without plastic is inconceivable, as plastic is highly versatile and can be

20 applied in various sectors ranging from the packaging to the building and construction sector

21 with a likewise wide range of lifetimes from less than a year to several decades. To date more

22 than 8.3 billion tonnes have been produced, out of which about 60% have been discarded. A

23 large proportion of the plastic waste is either disposed of in landfills or ends as litter in the

24 natural environment [1]. Hence plastic is not only advantageous, but also has become a

25 ubiquitous man-made environmental burden. Due to weathering and fragmentation, large

26 plastic items are gradually transformed to slowly degrading microplastic (MP) smaller than

27 5 mm [2, 3]. Although biodegradation by specialised microorganisms originating from plastic

28 polluted sites, landfills or animal intestines has been observed [4], MP only degrade slowly in

29 natural environments and persist over decades [5]. While most attention so far focused on

30 oceanic environments, the annual plastic release to terrestrial environments is 4-23 times

31 higher mostly due to agricultural practices and littering [6]. Conventional plastics, such as

32 polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride

33 (PVC), are considered to be extremely resistant to biodegradation. Biodegradable polymers

34 such as polylactic acid (PLA) and polycaprolactone (PCL) are therefore becoming popular as

35 an alternative. However, their degradation is incomplete and slow under environmental

36 conditions [7].

37 MP may not only alter the physical and chemical soil properties, but may also affect the soil

38 biota [8]. Unintentional ingestion of MP by soil-dwelling and especially soil-feeding macrofauna

39 is likely and can have negative fitness effects on organisms like earthworms, nematodes or

40 collembola [9, 10]. Subsequently MP may be transferred to higher trophic levels as shown for

41 chickens that acquired MP from soil by feeding on earthworms [11].

42 Alike earthworms, also terrestrial isopods (i.e., woodlice - Isopoda - Oniscidea) are widespread

43 decomposers with a density that can exceed 1000 individuals m^{-2} [12]. These organisms are

44 sensitive to contaminants, e.g. pesticides or heavy metals, and thus are suited for soil

45   ecotoxicity testing in laboratory and field bioindicator studies [12, 13]. As soil-dwellers they

46   mainly feed on decaying leaf litter and wood, and it has been shown that weathered feed

47   colonized by microbes is actually favoured [14, 15]. Their presence in soil enhances soil

48   nutrient cycling due to fragmentation and transportation of organic material along with

49   microorganisms [16]. Hence, it can be considered that they likewise contribute to the

50   fragmentation and transportation of accidently ingested MP and even distribution of pathogenic

51   microorganisms as plastic surfaces are suitable for colonization [17–19]. Many studies have

52   investigated the effects of pollutants on the isopod *Porcellio scaber* (common rough

53   woodlouse) [13]. However, to date there are only a few studies investigating life history traits

54   after MP ingestion: No or only minor effects on survival, feeding rate, body mass or energy

55   reserves in the digestive glands were obtained for isopods exposed to PE particles, tire

56   particles or polyester fibers [20–22]. However, this does not necessarily mean that *P. scaber*

57   is only marginally affected by MP ingestion. The effects are possibly less obvious after

58   relatively short exposure of only a few weeks and the detection of sublethal effects requires

59   assessment of other parameters. Accordingly, immune response parameters are affected by

60   MP (polyester fibers and tire particles) ingestion [23, 24]. Such effects are potentially linked to

61   effects on the gut microbiome, but the effects of MP exposure on the gut microbiome of *P.*

62   *scaber* have not been investigated yet.

63   Generally, an intact gut microbiome is important for the development, nutrition and immunity

64   and this also applies for isopods that possess a more dynamic microbiome compared to higher

65   organisms, such as mammals [25]. The nutrient content in the common diet of isopods (leaf

66   litter and dead wood) is generally very low and it is suggested that isopods rely on microbes

67   colonizing and degrading the decaying plant material and thereby providing nutrients for the

68   host, or the microbes themselves are digested and serve as nutrient-rich source [15, 26, 27].

69   With a reduced microbial cell number, it is likely that the latter applies for the anterior section

70   of the hindgut, while microbes proliferate towards the posterior section, where mainly

71   anaerobic conditions prevail [28–30]. The most abundant groups in *P. scaber's* gut microbiome

72   have been assigned to Proteobacteria, Bacteroidota, and Actinobacteria commonly inhabiting

73   insect intestines [26, 31–33]. All of these phyla contain members that possess a facultative or

74   even obligate anaerobic lifestyle and therefore, fermentative microbes including hydrogen

75   producers may play an important role as shown for other invertebrates (e.g., termites and

76   earthworms) [34, 35]. However, whether or not fermentation is an ongoing process in the gut

77   of *P. scaber* remains to be determined.

78   The gut microbial community and hence the digestive processes can indeed be modulated by

79   MP ingestion as shown for several soil invertebrates: In the gut of mealworms (*Tenebrio molitor*

80   larvae), PE and PS can be degraded with strong association of species within the

81   *Enterobacteriaceae* [36]. Adverse effects of MP ingestion on life history traits (e.g., reduced

82   growth and reproduction rates) appear along with alteration of the gut microbiome of springtails

83   (*Folsomia candida;* ingestion of PVC or PE) [37, 38] and potworms (*Enchytraeus crypticus;*

84   ingestion of PS) [39]. Studies of *P. scaber's* gut microbiome after MP ingestion are lacking to

85   date.

86   This study aims to investigate the effects of conventional non-biodegradable MP particles, PET

87   and PS, and biodegradable PLA on *P. scaber* with respect to fitness, gut microbiome and

88   fermentation potential in the gut, with the underlying hypothesis that biodegradable and non-

89   biodegradable MP have contrasting effects on *P. scaber* mediated by changes of the

90   associated gut microbiome. This was tested in MP-feeding experiments, microsensor analysis

91   of prevailing conditions in the gut with respect to pH, oxygen and hydrogen (as a measure for

92   ongoing microbial fermentation) concentration and analysis of the gut microbiome. In addition,

93   the food microbiome was analysed to investigate its influence on the gut microbiome.

94   **Materials and Methods**

95   *Food preparation and isopod collection*

96   Food pellets consisting of withered leaves (mainly maple leaves; 42%), ground commercial

97   rabbit food (25%) and potato powder (33%) were prepared as described in Žižek *et al.* [40].

98   For the pellets that additionally contained MP particles, 2.5% or 5% (w/w) PLA (NatureWorks,

99   Naarden, The Netherlands), PET (Veolia, Berlin, Germany) or PS (Ineos Styrolution,

100   Ludwigshafen, Germany) was added to the mixture. Granules were ground to fragments using

101   a cryo ball mill (Retsch, CryoMill, Germany) followed by sieving to obtain fragments ranging

102   from 75-150 µm in diameter of irregular shape prior usage.

103   *P. scaber* individuals (only adults; weight >30 mg) as model isopods were collected in a garden

104   near the campus of the University of Bayreuth (Germany) or the Leibniz University of Hannover

105   (Germany) between February and May 2020. The animals were kept in boxes (40 cm x 30 cm

106   x 25 cm) filled with damp soil, leaves, and tree bark prior performance of independent

107   experiments assessing hydrogen and methane emission rates of whole isopods, microsensor

108   profiles of pH, hydrogen and oxygen concentrations of isopod guts, bacterial community

109   composition of the isopod guts and food pellets, as well as fitness effects and food choice (for

110   the latter see Supplementary Material and Methods).

111   *Molecular hydrogen, oxygen and pH microsensor measurements from isopod guts*

112   Microsensor measurements were performed to identify the location and level of hydrogen

113   production within isopods as an estimate for the fermentation potential and to assess MP

114   effects on the conditions inside the gut of the isopods. 1 g of food pellets containing no MP or

115   5% PLA, PET or PS were mixed with 2 ml 1% agar (~60°C), spread on a petri dish and cooled

116   to room temperature. Twelve isopods per treatment were placed on these petri dishes and

117   kept at room temperature in the dark. The food was exchanged after 3 days and isopods were

118   kept for 3 further days. Prior to gut dissection, the isopods were placed on ice for several

119   minutes in order to lower their mobility. Each gut was embedded within a small glass chamber

120   in 1% low-melt agarose in insect Ringer's solution. Coverslips and microscope slides (7.5 x

121   2.5 x 0.1 cm) were used for construction of chambers similar to those in Brune *et al.* [41]: A

122   coverslip at the bottom of the chamber and two microscope slides on top of each other were

123   arranged to each side of the chamber providing the dimensions of 2.5 cm length, 1.0 cm width

124   and 0.2 cm depth. The bottom of the chamber was filled with a layer of molten 1% low melt

125   agarose in insect Ringer's solution and after solidification a freshly dissected full isopod gut

126   was placed on top of it. Then a top agarose layer (not warmer than 40°C) was cast in the

127   chamber, which was immediately covered with a coverslip before solidification. The embedded

128   gut was placed on another 2-mm thick agarose bed in a weighing boat and covered with insect

129   Ringer's solution.

130   Custom made microsensors for oxygen, hydrogen and pH [34, 42–44] with tip diameters

131   <20 µm were used for recording radial profiles of the anterior (at a distance of 1 mm from the

132   front end), the median and the posterior (at a distance of 1 mm from the rear end) of isopod

133   guts (see positions in Fig. S1). Measurements were performed at room temperature. For pH

134   measurements, a bridge consisting of a syringe barrel filled with the same agarose as the

135   agarose bed was constructed between this bed and a Red Rod reference electrode. The

136   sensors were connected to a four-channel multimeter with a built-in 16-bit A/D converter

137   (Unisense Microsensor Multimeter, Ver 2.01; Unisense A/S, Denmark). Pre-polarized sensors

138   were calibrated prior gut profile measurements: a two-point calibration with a 0.7 M alkaline

139   ascorbate solution (0 µM oxygen) and an air-saturated Ringer's solution (265.6 µM oxygen)

140   for the oxygen microsensor; a three-point calibration with pH 4, 7 and 10 buffers for the pH

141   microsensor; a calibration with multiple points ranging between 0 and 50 µM for the hydrogen

142   microsensor. Data acquisition and control of the micro-profiling system was enabled with the

143   software program SensorTrace PRO (Unisense A/S, Denmark). Profiling through the guts was

144   performed with 50 µm spatial resolution. Oxygen and pH sensors were allowed to equilibrate

145   for 5 s prior data acquisition. For hydrogen sensors, 10 s equilibration time were required. The

146   measurements with the different sensors were performed with different guts. Three guts per

147   treatment and microsensor were analysed at three positions each (anterior, median and

148   posterior).

149   *Determination of molecular hydrogen and methane emission from whole isopods*

150   In order to confirm hydrogen emissions under *in vivo* conditions, hydrogen production rates of

151   whole isopods were determined. In addition to hydrogen, methane is relevant in the anaerobic

152   food chain and was also analyzed. Therefore, adult isopods were collected and surface

153   sterilized with 70% ethanol. These animals were not subjected to a MP-treatment. Groups of

154   three individuals each were placed in three 3-mL Exetainer (Labco, Lampeter, UK). The vials

155   were sealed with airtight lids (caps with butyl septa) and overpressure was applied to all vials

156   via injection of 2 ml air. Headspace hydrogen and methane mixing ratios were analysed for a

157   period of 10 h with a gas chromatograph coupled to a pulsed discharge helium ionization

158   detector as described (7890B, Agilent Technologies, JAS GC systems, Moers, Germany) [45].

159   *Isopod feeding experiment with assessment of fitness parameters*

160   Groups of 10 isopods (7 females, 3 males) were kept in glass jars (diameter: 10.8 cm; volume:

161   370 ml) with the bottom covered with moist filter paper in a climate chamber with a 16 h light

162   and 8 h dark circle at 16°C and 85% humidity. Isopod groups were exposed to food pellets

163   without or with 2.5% or 5% PLA, PET or PS for eight weeks. Each treatment was performed in

164   5 replicates. One food pellet was placed in each jar and exchanged every other day. At the

165   same time survival of isopods was checked and dead individuals were removed. Once a week

166   the glass jars were cleaned and the filter papers were replaced. Locomotor activity tests were

167   performed after two, four and six weeks (see Supplementary Material and Methods). After eight

168   weeks, the isopods were weighed and the percentage weight gain to the initial weight was

169   calculated. Further, one gut per replicate was dissected and these guts as well as the food

170   pellet of the respective treatment were frozen in liquid nitrogen and kept at -80°C prior

171   microbiome analysis.

172   *Nucleic acid extraction, DNase treatment and reverse transcription*

173   Prior extraction of DNA and RNA the weight of the whole isopod guts was determined. The

174   nucleic acids were also extracted from subsamples of the food pellets (~50 mg). The extraction

175   protocol was performed according to Griffiths *et al.* [46] with some modifications: i) 2-ml screw

176   cap tubes used during initial cell lysis were filled with Ø0.1-mm and Ø0.5-mm zirconium beads,

177   150 mg each, and one Ø3-mm glass bead; ii) cell lysis by bead beating for 30 s at 5.0 m s^{-1}

178   was performed twice with an intermitted cooling on ice for 30 s; iii) nucleic acids were

179   precipitated on ice for 2 h; iv) air-dried nucleic acid pellets derived from gut and food samples

8

180   were resuspended in 30 μl and 60 μl RNase-free water, respectively. Verification of nucleic

181   acid extracts was assessed via agarose gel electrophoresis and spectrophotometric

182   measurements. DNase treatment was applied to a 13-μl subsample of each extract using the

183   TURBO DNA-free™ Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) according

184   to the manufacturer's instructions. Reverse transcription of 10 μl RNA was performed using

185   LunaScript RT Supermix Kit (New England Biolabs, Ipswich, MA, USA) according to the

186   manufacturer's protocol. Negative controls without template RNA (water treated with DNase)

187   and, for each sample, without reverse transcriptase were performed. Samples were kept on

188   ice for further processing.

189   *Analysis of bacterial 16S rRNA genes and 16S rRNA*

190   Bacterial 16S rRNA genes and 16S rRNA (after reverse transcription) were quantified by qPCR

191   using primers Bact_341F and Bact_805R [47]. Each sample was analyzed in duplicate 10-μl

192   reactions containing 5 μl Luna Universal qPCR Master Mix (New England Biolabs, Ipswich,

193   MA, USA), 2 μM of each primer, 10 μg bovine serum albumin and 2 μl of 1:100 diluted template

194   (c)DNA. Negative controls contained sterile water instead of template (c)DNA. Standards

195   consisted of serially diluted (10^2 to 10^6 gene copies per μl) M13uni/rev PCR products of a

196   pGEM-T vector with a 16S rRNA gene inserted. The amplification was performed in a CFX

197   Connect Real-Time PCR System (Bio-Rad, Feldkirchen, Germany) with the following cycling

198   conditions: 2 min at 95°C, 35 cycles of 30 s at 95°C, 50 s at 60°C (combined annealing and

199   elongation) and a plate read after 10 s at 80°C. The amplification efficiencies ranged between

200   96% and 98%, the r² values were ≥0.99. The specificity of the amplification was verified by

201   melting curve analysis (from 60°C to 95°C in 0.5°C-intervals for 5 s each) and agarose gel

202   electrophoresis. Specific amplification of archaeal 16S rRNA (using primers A519F [48] and

203   A1017R [49]) and [Fe-Fe]-hydrogenase genes (encoding for enzymes that catalyze the

204   production of hydrogen in obligate anaerobic fermenting bacteria, using primers from Schmidt

205   *et al.* [50] and Xing *et al.* [51]) was tested for several gut and food samples, but consistently

206   failed.

207 Bacterial 16S rRNA genes and 16S rRNA were also analyzed by high-throughput amplicon

208 sequencing. Amplicons were generated using the same primer pairs as for the qPCR, but

209 tagged with specific adapters (Illumina, San Diego, USA). Each reaction mixture contained

210 12.5 µl Kapa HiFi HotStart ReadyMix (Roche, Mannheim, Germany), 0.5 µM of each primer,

211 5 µg bovine serum albumin and 2.5 µl of 1:10 diluted template (c)DNA. Sterile water, instead

212 of template was applied for negative controls. The PCR was performed in a Thermocycler

213 (Biozym Scientific GmbH, Hessisch Oldendorf, Deutschland) with 3 min initial denaturation at

214 95°C, followed by 30 cycles with 20 s at 98°C, 15 s at 55°C and 15 s at 72°C, and an end-

215 elongation step at 72°C for 1 min. Specificity of the amplification was confirmed by agarose

216 gel electrophoresis. The amplicons were purified using the GeneRead size selection kit

217 (Qiagen, Hilden, Germany). Library preparation was performed using the Nextera XT Index Kit

218 (Illumina, San Diego, United States) as given elsewhere [52] and the Illumina MiSeq version 3

219 chemistry was applied for 2x300 bp paired-end sequencing. Details regarding generation of

220 amplicon sequencing variants (ASVs) and further analyses of the sequences are provided in

221 the Supplementary Material and Methods.

222 *Statistical analysis*

223 All statistical analyses were conducted using the statistical platform R version 4.1.1 [53].

224 Differences in weight gain, food choice and speed index of the isopods as well as maximum

225 hydrogen concentration and minimum pH in the isopod guts were analyzed by fitting linear

226 models, performing ANOVA tests and Tukey *post hoc* comparisons using the *multcomp*

227 package [54]. The survival probability of isopods and confidence intervals were calculated

228 using the *survival* package [55]. Abundance data obtained from qPCR analysis was

229 investigated by a factorial two-way ANOVA (MP-treatment and percentage as factors). If the

230 initial model has not met normality assumptions, the data was transformed using the

231 transformTukey function in the *rcompanion* package [56], and then the adjusted model met the

232 required assumptions. Tukey *post hoc* tests were applied to assess significant differences in

233 means. Whether the community compositions differed between the treatments was assessed

234 with PERMANOVA tests and pairwise comparisons applied on the Aitchison distance matrix

235 using the adonis2 and the adonis.pair function in the *vegan* [57] and *EcolUtils* [58] packages

236 of R, respectively.

Results

Effects of microplastic on isopods

239 When *P. scaber* had the choice between food containing no MP or 5% PLA, PET or PS, a

240 significant avoidance of PS-food was observed (Fig. S2a). However, when there was no choice

241 given, any food was ingested. Neither the survival (~20% dead individuals after eight weeks;

242 Fig. S3), nor the weight gain was affected by the MP-diet (p = 0.556; Fig. S2b). The speed

243 index for isopods exposed to 2.5% PET was significantly lower than that of those exposed to

244 2.5% PS, nevertheless none of the MP-food had a significant effect compared to the control-

245 food (Fig. S2c).

Physicochemical conditions in the gut of isopods and effects of microplastic ingestion

247 Radial oxygen micro-profiles revealed anoxic conditions at any position in the guts of isopods

248 fed with any diet, and pH ranged from 5 to 7 (Figs. S4, S5). Minimum gut pH values were not

249 affected by the MP-treatment nor an interaction of position and MP-treatment (ANOVA; p >

250 0.05). The minimum pH was significantly more acidic in the anterior (pH 5.2) than in the median

251 to posterior (pH 5.8) positions (Fig. S5, Table S1). Hydrogen was highest inside the isopod

252 guts at all measured positions (Figs. 1, S6). Hydrogen concentrations of up to ~20 µM were

253 detected in the gut center of isopods fed with MP-free control food. Isopods fed with PLA-food

254 showed highest and those fed with PET- or PS-food lowest gut hydrogen concentrations. At

255 the gut median, maximum hydrogen concentrations of ~30 µM were significantly higher in

256 isopods fed with PLA-food than with other food (Table. S2). At the posterior position of the gut,

257 maximum hydrogen concentrations of ~5 µM were significantly lower in isopods fed with PET-

258 and PS-food than with control- and PLA-food. It is also worth mentioning that in guts of isopods

259   fed with control- and PLA-food, the hydrogen formation activity was significantly higher towards

260   the posterior than in the anterior end.

261   *Hydrogen emission potential of whole isopods*

262   Hydrogen mixing ratios in the headspace of vials containing whole isopods (analyzed directly

263   after collection and not subjected to MP-treatments) increased linearly over time without

264   appreciable delay (Fig. 2), demonstrating that hydrogen is indeed emitted from whole isopods

265   under *in vivo* conditions. The emission rates were highly variable with an average rate of 0.83

266    $\pm$ 0.51 ng hydrogen isopod^{-1} h^{-1} resulting in a final amount between 1 and 24 ng hydrogen

267   isopod^{-1} after 10 hours of incubation.

268   *Impact of microplastic on 16S rRNA gene and 16S rRNA abundance in food and guts of*

269   *isopods*

270   Bacterial 16S rRNA abundances were essentially one order of magnitude higher in the isopod

271   guts than in the food pellets, while the opposite applied for 16S rRNA genes (Fig. 3). Such

272   findings were supported by the 16S rRNA:16S rRNA gene ratios that were consistently higher

273   in the guts than in the food despite a high variability among replicates (Fig. 3c,f). 16S rRNA

274   gene abundances obtained from the guts were significantly higher in isopods exposed to PLA-

275   food compared to the other treatments (Fig. 3a). A similar stimulation was reflected at

276   16S rRNA level, but not in 16S rRNA:16S rRNA gene ratios (Fig. 3b,c). MP had no significant

277   effects on the 16S rRNA gene and 16S rRNA abundances in the food pellets and ratios thereof

278   (Fig. 3d,e,f).

279   *Impact of microplastic on the bacterial communities*

280   The bacterial communities in the isopod guts were highly diverse (Fig. S8c,d). Highest relative

281   gene abundances were found for taxa within the Actinobacteria (mainly *Microbacteriaceae*),

282   Bacteroida (mainly *Flavobacteriaceae*), Gammaproteobacteria (mainly *Enterobacteriaceae*

283   and *Vibrionaceae*) and Verrucomicrobiae (mainly *Opitutaceae*) (Fig. S8c). All of these taxa,

284   but the Actinobacteria to a minor extent, were also found at 16S rRNA level (Fig. S8d).

285   Gut communities differed from the food communities as indicated by principal coordinates

286   analysis (PCoA; PERMANOVA; $p < 0.005$; Fig. S9a; for further details see Supplementary

287   Results). ASVs assigned to *Vibrio rumoiensis* correlated well with gut communities. To

288   elucidate the effects of MP, the datasets were analyzed separately for the gut and the food

289   communities (Figs. 4, S9b). In addition to *Vibrio rumoiensis*, the gut communities were mainly

290   affected by *Enterobacteriaceae* and *Microbacteriaceae* (Fig. 4). PERMANOVA analysis

291   revealed that gut communities differed significantly due to MP treatment. An effect of MP

292   treatment and dosage was obtained. For the latter, significant difference between communities

293   exposed to 2.5% and 5% MP has been confirmed by a pairwise comparison (Table S3). Such

294   pairwise comparisons essentially confirmed differences between gut communities of isopods

295   fed with PLA-food and those fed with PET- or PS-food with low p-values ($p < 0.07$), but failed

296   to confirm other MP treatment effects ($p > 0.15$) (Table S4).

297   *Shared, unique and indicator taxa with respect to MP-treatments*

298   Most genera were shared among all treatments in isopod guts (43-49%; Figs. 5, S10a). On

299   16S rRNA gene and 16S rRNA level, only a few genera were uniquely found in guts of isopods

300   fed with PET- or PS-food (2-4%), but more in case of guts of isopods fed with control- or PLA-

301   food (7-13%). Moreover, more genera in guts of isopods exposed to control-food were shared

302   with those exposed to PLA-food (8-13%) than with those exposed to PET- (1%) or PS-food

303   (0%). Genera within *Alcaligenaceae* were among exclusive taxa in guts of PLA-food exposed

304   isopods.

305   Some taxa were also found to be significantly indicative in these guts (Fig. 6; Tables S6, S7).

306   The majority of indicator genera was found in guts of isopods fed with PLA-food (8 out of 14

307   and 13 out of 21 genera on 16S rRNA gene and 16S rRNA level, respectively) and none were

308   found in those fed with PS-food. The communities in guts of isopods fed with control-food were

309   more similar to those fed with PLA-food than to those fed with PET- or PS-food, as the former

13

310 shared more indicator taxa. In particular, most of these genera were more abundant in guts of

311 isopods fed with PLA-food than in those fed with control-food. *Chryseobacterium*, *Devosia*,

312 *Niabella, Prosthecobacter*, *Taeseokella* and uncultured Rhodospirillales were among the

313 indicator taxa on 16S rRNA level in guts of isopods fed with PLA-food (Fig. 6a, Table S7). In

314 guts of isopods fed with PET-food, *Legionella*, *Microbacterium*, *Mycobacterium*, *Paenibacillus*

315 and at least three different genera within the *Enterobacteriaceae* were attributed to indicator

316 genera on 16S rRNA level (Fig. 6b, Table S7).

317 In addition, MP affected food communities. More exclusive genera were obtained in the PS-

318 food than in other kind of food-pellets (Fig. S10b,c). Accordingly, more indicator taxa were

319 found in the PS-food with most of them belonging to the Rhizobiales (Tables S8 and S9). For

320 the control- or PLA-food, no indicators were found. Notably, none of the MP-specific indicator

321 taxa found in the guts were reflected in the respective food-pellets (Tables S7-S10). Such a

322 finding was supported by comparing the abundance of MP-specific indicator taxa from the guts

323 with their abundance in the food pellets (Figs. 6, S11, S12).

324 **Discussion**

325 Isopods are globally abundant detritivores with a rarely studied gut environment and

326 microbiome, as well as an unknown relevance for atmospheric trace gas emissions. Effects of

327 MP pollution on model detritivores are largely unclear to date. Thus, we provide new insights

328 into effects of biodegradable (PLA) and non-biodegradable (PS and PET) MP polymer types

329 on the gut microbial community as well as activity of the model isopod *P. scaber*, and identify

330 *P. scaber* as a MP-impacted mobile source of molecular hydrogen. We extend previous studies

331 on the effect of PE, tire particles or polyester fibers on life history traits in *P. scaber* that

332 revealed no or only marginal fitness effects [20–22], which is in line with the findings of this

333 study. *P. scaber* was not affected by the ingestion of MP with its food as neither mortality nor

334 weight gain or locomotor activity were altered (Figs. S2, S3). However, when given a choice

335 between control- and MP-food the isopods significantly avoided food containing PS. The

336 palatability of the food source coheres with its microbial composition [14, 15] and therefore it

14

337   can be considered that PS-food was less attractive, as indeed, most differences of the

338   microbial composition were found between control- and PS-food (Fig. S9). Avoidance

339   behaviour of isopods is commonly observed against metals, pesticides, pharmaceuticals or

340   chars, and already at low concentrations, it is often a more sensitive measure for adverse

341   effects compared to fitness parameters [59–63]. Effects of long-term exposure to MP-

342   contaminated food sources on the isopods' fitness have not been addressed and cannot be

343   excluded.

344   Despite the importance of the gut microbiome in soil invertebrates, previous studies testing MP

345   effects on invertebrates are limited [36–39] and are absent in the case of *P. scaber*. In this

346   study, analyses were performed on 16S rRNA gene and 16S rRNA level with the former

347   reflecting the present community and the latter the rather active part of this community, which

348   is commonly a more sensitive response measure. Findings regarding the general gut and food

349   microbiome (MP treatment independent) are discussed in the Supplementary Discussion. The

350   bacterial gut communities of isopods exposed to MP did not differ significantly from the control

351   according to pairwise comparisons (Table S4), but there were differences with respect to

352   exclusive and indicative taxa that were not related to the food communities (Figs. 5, 6, S10-

353   S12, Tables S6-S9). Generally, the gut communities of the isopods fed with MP-free control-

354   food shared more taxa and indicators with those fed with PLA-food than with PS- or PET-food.

355   Moreover, PLA-food increased bacterial proliferation as growth (16S rRNA gene level) and

356   activity (16S rRNA level) were stimulated (Fig. 3). This suggests that some PLA has been

357   degraded in the isopod guts. Abiotic degradation of PLA occurs due to hydrolysis of ester

358   linkages releasing lactic acid at pH 4-7 at low rates [64–66]. The moderately acidic pH inside

359   the gut would thus allow for some abiotic, acid-catalyzed PLA hydrolysis. However, such

360   abiotic hydrolysis takes several months or even years at environmentally relevant

361   temperatures (<30°C) [67]. Biotic PLA degradation is enhanced by enzymatic cleavage of ester

362   bonds and depolymerization of the polymer to oligomers, dimers and lactic acid monomers

363   [68]. Various taxa possess hydrolytic, PLA depolymerizing enzymes like certain lipases,

364   carboxylesterases, and proteinases [69, 70]. Many of such enzymes are active at the gut pH

15

365   of greater than or equal to 5 [69, 71], suggesting microbial, enzyme-catalyzed rather than

366   abiotic PLA hydrolysis to lactate in the gut of isopods. Subsequent fermentation of lactate in

367   the anoxic gut environment is likely [72, 73].

368   Indeed, some genera within the Actinobacteria ubiquitous in the isopod guts and less abundant

369   in the food pellets (Fig. S8; Supplementary Discussion) are possible PLA degraders, as

370   numerous members of this class are capable of PLA degradation [74]. Actinobacteria include

371   aerobes as well as anaerobes and may subsist in the mainly anoxic isopod guts [75]. Members

372   of *Alcaligenaceae* were exclusively found in the guts of isopods fed with PLA-food. *Alcaligenes*

373   sp. of the *Alcaligenaceae* are well known to produce lipases that might contribute to PLA

374   hydrolysis [75, 76]. The absence of a massive stimulation of such taxa in PLA treatments in

375   spite of a potential PLA hydrolysis activity might be due to the lack of a specialized metabolism

376   necessary for energy conservation from lactate under anoxic conditions. *Saprospiraceae*

377   (*Parapedobacter*), *Micavibrionaceae* and *Saccharimonadaceae* (TM7) were indicators for guts

378   of isopods exposed to PLA-food (Table S6). *Parapedobacter luteus* of the *Saprospiraceae* is

379   capable of Tween 80 hydrolysis, which has structural similarities to PLA [77]. *Micavibrionaceae*

380   showed affinity for PLA-blended PBAT films [78], suggesting a possible role of both taxa in PLA

381   hydrolysis. *Saccharimonadaceae* (also known as Saccharibacteria, TM7 or clade G6) show an

382   anaerobic lifestyle, were enriched in soil with the structurally related polymer PBAT, are

383   proposed to scavenge small molecular weight carbon during hydrocarbon degradation and

384   host lactate dehydrogenases, suggesting their involvement in lactic acid removal during PLA

385   degradation [79–82]. Additionally, *Xanthomonadaceae* were found in an anaerobic sludge

386   incubation supplemented with PLA [83], suggesting that *Pseudoxanthomonas,* an active genus

387   indicative for guts of PLA-exposed isopods, may have contributed to PLA degradation (Fig. 6).

388   Further most abundant indicators here were *Chryseobacterium*, *Devosia*, *Niabella*,

389   *Prosthecobacter*, *Taeseokella* and uncultured Rhodospirillales. Potential PLA degradation

390   capabilities of these taxa is currently unknown, but cannot be excluded and they may also

391   possess enzymes capable of cleaving the ester bonds of PLA. However, they may have also

392   taken advantage from enhanced lactic acid release during PLA degradation. A facultative

393   lifestyle is conceivable for all of them [84–89].

394   A further novelty of this study was the assessment of *in situ* hydrogen production in the isopod

395   guts via microsensor measurements. So far such hydrogen production has only been shown

396   for two other soil dwelling invertebrates, the earthworm *Lumbricus terrestris* and the termite

397   *Reticulitermes flavipes* [34, 35]. Hydrogen production in the guts of isopods exposed to PLA-

398   food was higher than in the other guts (Figs. 1, S6, Table S1), suggesting higher fermentative

399   activities in presence of PLA. Degradation of PLA generates lactate (see above), which can

400   then be fermented to acetate, propionate, carbon dioxide and water, or hydrogen [72, 73].

401   Obligate anaerobes like *Clostridiaceae* are capable of fermenting acetate and propionate to

402   hydrogen and carbon dioxide, but were absent from isopod guts (Fig. S8). Moreover, other

403   hydrogen-producing fermenting gut bacteria, that are often obligate anaerobes [50], were not

404   identified by the sequencing analyses (Fig. S8) and [Fe-Fe]-hydrogenase genes (in the

405   genomic repertoire of these organisms) were not PCR-amplifiable. The absence of obligate

406   anaerobes in the gut system is somewhat surprising compared to other anoxic gut systems

407   [34, 35, 90, 91] and probably owed to the short gut passage time of ~5 h not allowing for the

408   proliferation of such organisms from ingested inactive forms [92]. However, the short gut

409   passage time will suffice for the activation of fermentation by facultatives. *Enterobacteriacea*

410   are facultatives, well known to produce hydrogen via mixed acid fermentation. An explanation

411   for the stimulated hydrogen production in PLA-fed isopod guts is thus a variation of the mixed

412   acid fermentation pathway by *Enterobacteriaceae* generating ethanol, acetate and formate,

413   and associated formate hydrogen lyase (FHL) catalyzed hydrogen production (Fig. 7) [93]. A

414   'proof of principle' experiment using *Escherichia coli* as a model organism of the

415   *Enterobacteriaceae* has indeed revealed that hydrogen was generated from lactate (see

416   Supplementary Information for Material and Methods and Results; Fig. S13). A pH of 5 to 6 in

417   the median part of the gut, where hydrogen production was high (Figs. 1, S5, S6), represents

418   favourable conditions for FHL activity and formate transformation [93]. *Enterobacteriacea* were

419   ubiquitous as well as active in *P. scaber* guts as was hydrogen production. Energy

420 conservation via the variation of the mixed acid fermentation pathway is only little not leading

421 to detectable stimulation in growth or activity and may explain why *Enterobacteriacea* were not

422 identified as indicators for the PLA-fed isopod guts.

423 In contrast, little hydrogen was produced in the guts of isopods fed with PET- and PS-food.

424 Hydrogen consumption by obligate anaerobic methanogens was unlikely to be the reason, as

425 amplification targeting archaeal 16S rRNA failed and methane was not produced (data not

426 shown), when whole isopods were analysed. However, two reasons are conceivable: Either

427 the MP had inhibitory effects on fermentative microorganisms, or Knallgas bacteria were

428 stimulated and consumed most of the hydrogen. Some evidence is given for the latter:

429 *Mycobacterium*, an indicator for the gut of isopods fed with PET-food (Table S8), has been

430 identified as a hydrogen-oxidising bacterium with oxygen as electron acceptor [94]. Oxygen

431 diffusing in the isopod guts from the gut wall might enable hydrogen consumption leading to

432 immediate consumption like well known from termites [41]. Nevertheless, the actual reason of

433 reduced hydrogen emission remains to be determined.

434 Hydrogen is a valuable electron donor fueling hydrogen-oxidizing processes either inside or

435 outside the gut and MP contamination may have consequences for microbial food webs and

436 global hydrogen emissions. *P. scaber* hydrogen concentrations in the center of the gut ranged

437 from 5 to 30 µM and were thus in the range of those from *L. terrestris* and *R. flavipes*,

438 demonstrating *P. scaber*'s high hydrogen emission potential. Hydrogen emissions from whole

439 isopods were variable, on average 0.83 ± 0.51 ng isopod^{-1} h^{-1} (Fig. 2). Assuming that 20% of

440 the Earth's terrestrial ecosystems (total surface area of 1.5×10^{14} m^2 [95]) are colonized by

441 these cosmopolitan isopods with a density of 75 isopods m^{-2} (median of distributions given in

442 Paoletti and Hassall [12]), the annual contribution of *P. scaber* to the global hydrogen

443 production is approximately 0.6 to 2.6×10^7 kg yr^{-1}. This value is in the same range of what is

444 annually emitted from paddy fields (1.3×10^7 kg yr^{-1}) [96].

445 Taken together, this study provides new insights regarding the effects of MP on soil

446 invertebrates that are potentially affected by MP-ingestion in the longer term and highlights the

447   hitherto unknown hydrogen emitting capacity of a widely distributed group of detritivores. We

448   identified the moderately acidic, anoxic, median and posterior parts of the isopod's gut as 'hot

449   spots' for hydrogen production. Such a hydrogen production was stimulated by PLA- and

450   inhibited by PET- and PS-ingestion, which was concomitant to changes in the composition of

451   the gut microbiome and in agreement with our initial hypothesis that biodegradable and non-

452   biodegradable MP have contrasting effects. The nature of low hydrogen emissions in response

453   to PET and PS exposure remains speculative, as are consequences of altered hydrogen

454   metabolism inside and outside the isopod gut, opening up new avenues for future research.

463   **Competing Interests**

464   The authors declare no competing interests.

465   **Data availability**

466   Amplicon sequencing data have been deposited in the NCBI Sequence Read Archive

467   (https://www.ncbi.nlm.nih.gov/sra) under the Bioproject PRJNA832915.

References

1. Geyer R, Jambeck JR, Law KL. Production, use, and fate of all plastics ever made. Sci Adv, 2017; 3:e1700782.

2. Andrady AL. The plastic in microplastics: A review. Mar Pollut Bull, 2017; 119:12–22.

3. Hartmann NB, Hüffer T, Thompson RC, Hassellöv M, Verschoor A, Daugaard AE et al. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. Environ Sci Technol, 2019; 53:1039–1047.

4. Gambarini V, Pantos O, Kingsbury JM, Weaver L, Handley KM, Lear G. Phylogenetic distribution of plastic-degrading microorganisms. mSystems, 2021; 6:1–13.

5. Lebreton L, Egger M, Slat B. A global mass budget for positively buoyant macroplastic debris in the ocean. Sci Rep, 2019; 9:12922.

6. Horton AA, Walton A, Spurgeon DJ, Lahive E, Svendsen C. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci Total Environ, 2017; 586:127–141.

7. Emadian SM, Onay TT, Demirel B. Biodegradation of bioplastics in natural environments. Waste management, 2017; 59:526–536.

8. Büks F, Kaupenjohann M. The impact of microplastic weathering on interactions with the soil environment: A review. SOIL Discuss, 2022; preprint:1–22.

9. Büks F, van Loes Schaik N, Kaupenjohann M. What do we know about how the terrestrial multicellular soil fauna reacts to microplastic? SOIL, 2020; 6:245–267.

10. Ji Z, Huang Y, Feng Y, Johansen A, Xue J, Tremblay LA et al. Effects of pristine microplastics and nanoplastics on soil invertebrates: A systematic review and meta-analysis of available data. Sci Total Environ, 2021; 788:147784.

11. Huerta Lwanga E, Mendoza Vega J, Ku Quej V, Chi JdLA, Del Sanchez Cid L, Chi C et al. Field evidence for transfer of plastic debris along a terrestrial food chain. Sci Rep, 2017; 7:14071.

12. Paoletti MG, Hassall M. Woodlice (Isopoda: Oniscidea): Their potential for assessing sustainability and use as bioindicators. Agriculture, Ecosystems & Environment, 1999; 75:157–165.

497 13. van Gestel CAM, Loureiro S, Idar P. Terrestrial isopods as model organisms in soil
498   ecotoxicology: A review. Zookeys, 2018; 801:127–162.

499 14. Hassall M, Turner JG, Rands MRW. Effects of terrestrial isopods on the decomposition of
500   woodland leaf litter. Oecologia, 1987; 72:597–604.

501 15. Ihnen K, Zimmer M. Selective consumption and digestion of litter microbes by Porcellio
502   scaber (Isopoda: Oniscidea). Pedobiologia, 2008; 51:335–342.

503 16. Rabatin SC, Stinner BR. Indirect effects of interactions between VAM fungi and soil-
504   inhabiting invertebrates on plant processes. Agric Ecosyst Environ, 1988; 24:135–146.

505 17. Gkoutselis G, Rohrbach S, Harjes J, Obst M, Brachmann A, Horn MA et al. Microplastics
506   accumulate fungal pathogens in terrestrial ecosystems. Sci Rep, 2021; 11:13214.

507 18. Kirstein IV, Kirmizi S, Wichels A, Garin-Fernandez A, Erler R, Löder M et al. Dangerous
508   hitchhikers? Evidence for potentially pathogenic Vibrio spp. on microplastic particles. Mar
509   Environ Res, 2016; 120:1–8.

510 19. Rohrbach S, Gkoutselis G, Hink L, Weig AR, Obst M, Diekmann A et al. Microplastic
511   polymer properties as deterministic factors driving terrestrial plastisphere microbiome
512   assembly and succession in the field (in prep.).

513 20. Jemec Kokalj A, Horvat P, Skalar T, Kržan A. Plastic bag and facial cleanser derived
514   microplastic do not affect feeding behaviour and energy reserves of terrestrial isopods.
515   Sci Total Environ, 2018; 615:761–766.

516 21. Selonen S, Dolar A, Jemec Kokalj A, Sackey LNA, Skalar T, Cruz Fernandes V et al.
517   Exploring the impacts of microplastics and associated chemicals in the terrestrial
518   environment - Exposure of soil invertebrates to tire particles. Environ Res, 2021;
519   201:111495.

520 22. Selonen S, Dolar A, Jemec Kokalj A, Skalar T, Parramon Dolcet L, Hurley R et al.
521   Exploring the impacts of plastics in soil - The effects of polyester textile fibers on soil
522   invertebrates. Sci Total Environ, 2020; 700:134451.

523 23. Dolar A, Drobne D, Dolenec M, Marinšek M, Jemec Kokalj A. Time-dependent immune
524   response in Porcellio scaber following exposure to microplastics and natural particles. Sci
525   Total Environ, 2022; 818:151816.

24. Dolar A, Selonen S, van Gestel CAM, Perc V, Drobne D, Jemec Kokalj A. Microplastics, chlorpyrifos and their mixtures modulate immune processes in the terrestrial crustacean Porcellio scaber. Sci Total Environ, 2021; 772:1–11.

25. Bouchon D, Zimmer M, Dittmer J. The terrestrial isopod microbiome: An all-in-one toolbox for animal-microbe interactions of ecological relevance. Front Microbiol, 2016; 7:1472.

26. Horváthová T, Babik W, Bauchinger U. Biofilm feeding: Microbial colonization of food promotes the growth of a detritivorous arthropod. Zookeys, 2016; 577:25–41.

27. Zimmer M. Nutrition in terrestrial isopods (Isopoda: Oniscidea): An evolutionary-ecological approach. Biol Rev Camb Philos Soc, 2002; 77:455–493.

28. Drobne D. Bacteria adherent to the hindgut of terrestrial isopods. Acta Microbiol Immunol Hung, 1995; 42:45–52.

29. Zimmer M, Brune A. Physiological properties of the gut lumen of terrestrial isopods (Isopoda: Oniscidea): Adaptive to digesting lignocellulose? J Comp Physiol B, 2005; 175:275–283.

30. Zimmer M, Topp W. Microorganisms and cellulose digestion in the gut of the woodlouse Porcellio scaber. J Chem Ecol, 1998; 24:1397–1408.

31. Horváthová T, Babik W, Kozłowski J, Bauchinger U. Vanishing benefits - The loss of actinobacterial symbionts at elevated temperatures. J Therm Biol, 2019; 82:222–228.

32. Kostanjsek R, Lapanje A, Rupnik M, Strus J, Drobne D, Avgustin G. Anaerobic bacteria in the gut of terrestrial isopod crustacean Porcellio scaber. Folia Microbiol, 2004; 49:179–182.

33. Kostanjsek R, Strus J, Avgustin G. Genetic diversity of bacteria associated with the hindgut of the terrestrial crustacean Porcellio scaber (Crustacea: Isopoda). FEMS Microbiol Ecol, 2002; 40:171–179.

34. Ebert A, Brune A. Hydrogen concentration profiles at the oxic-anoxic interface: A microsensor study of the hindgut of the wood-feeding lower termite Reticulitermes flavipes (Kollar). Appl Environ Microbiol, 1997; 63:4039–4046.

35. Wüst PK, Horn MA, Drake HL. In situ hydrogen and nitrous oxide as indicators of concomitant fermentation and denitrification in the alimentary canal of the earthworm Lumbricus terrestris. Appl Environ Microbiol, 2009; 75:1852–1859.

556 36. Brandon AM, Gao S-H, Tian R, Ning D, Yang S-S, Zhou J et al. Biodegradation of
557 polyethylene and plastic mixtures in mealworms (larvae of Tenebrio molitor) and effects
558 on the gut microbiome. Environ Sci Technol, 2018; 52:6526–6533.

559 37. Ju H, Zhu D, Qiao M. Effects of polyethylene microplastics on the gut microbial
560 community, reproduction and avoidance behaviors of the soil springtail, Folsomia candida.
561 Environ Pollut, 2019; 247:890–897.

562 38. Zhu D, Chen Q-L, An X-L, Yang X-R, Christie P, Ke X et al. Exposure of soil collembolans
563 to microplastics perturbs their gut microbiota and alters their isotopic composition. Soil
564 Biol Biochem, 2018; 116:302–310.

565 39. Zhu B-K, Fang Y-M, Zhu D, Christie P, Ke X, Zhu Y-G. Exposure to nanoplastics disturbs
566 the gut microbiome in the soil oligochaete Enchytraeus crypticus. Environ Pollut, 2018;
567 239:408–415.

568 40. Zižek S, Hrženjak R, Kalcher GT, Srimpf K, Semrov N, Zidar P. Does monensin in chicken
569 manure from poultry farms pose a threat to soil invertebrates? Chemosphere, 2011;
570 83:517–523.

571 41. Brune A, Emerson D, Breznak JA. The termite gut microflora as an oxygen sink:
572 Microelectrode determination of oxygen and pH gradients in guts of lower and higher
573 termites. Appl Environ Microbiol, 1995; 61:2681–2687.

574 42. Revsbech NP. An oxygen microsensor with a guard cathode. Limnol Oceanogr, 1989;
575 34:474–478.

576 43. Revsbech NP, Jørgensen BB. Microelectrodes: Their use in microbial ecology. In:
577 Marshall K.C. (ed). Advances in Microbial Ecology, vol 9. Springer, Boston, MA, 1986. pp
578 293–352.

579 44. Schramm A. Microsensors for the study of microenvironments and processes in the
580 intestine of invertebrates. In: König H, Varma A (eds). Intestinal Microorganisms of
581 Termites and Other Invertebrates. Springer, Berlin, Heidelberg, 2006. pp 463–473.

582 45. Zaman M, Kleineidam K, Bakken L, Berendt J, Bracken C, Butterbach-Bahl K et al.
583 Automated laboratory and field techniques to determine greenhouse gas emissions. In:
584 Zaman M, Heng L, Müller C (eds). Measuring Emission of Agricultural Greenhouse Gases
585 and Developing Mitigation Options using Nuclear and Related Techniques. Springer
586 International Publishing, Cham, 2021. pp 109–139.

587 46. Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ. Rapid method for coextraction of DNA
588   and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based
589   microbial community composition. Appl Environ Microbiol, 2000; 66:5488–5491.

590 47. Herlemann DP, Labrenz M, Jürgens K, Bertilsson S, Waniek JJ, Andersson AF.
591   Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea.
592   ISME J, 2011; 5:1571–1579.

593 48. Wang Y, Qian P-Y. Conservative fragments in bacterial 16S rRNA genes and primer
594   design for 16S ribosomal DNA amplicons in metagenomic studies. PLOS ONE, 2009;
595   4:e7401.

596 49. Yoshida N, Yagi K, Sato D, Watanabe N, Kuroishi T, Nishimoto K et al. Bacterial
597   communities in petroleum oil in stockpiles. J Biosci Bioeng, 2005; 99:143–149.

598 50. Schmidt O, Drake HL, Horn MA. Hitherto unknown Fe-Fe-hydrogenase gene diversity in
599   anaerobes and anoxic enrichments from a moderately acidic fen. Appl Environ Microbiol,
600   2010; 76:2027–2031.

601 51. Xing D, Ren N, Rittmann BE. Genetic diversity of hydrogen-producing bacteria in an
602   acidophilic ethanol-H2-coproducing system, analyzed using the Fe-hydrogenase gene.
603   Appl Environ Microbiol, 2008; 74:1232–1239.

604 52. Ho A, Mendes LW, Lee HJ, Kaupper T, Mo Y, Poehlein A et al. Response of a methane-
605   driven interaction network to stressor intensification. FEMS Microbiol Ecol, 2020;
606   96:fiaa180.

607 53. R Core Team. R: A language and environment for statistical computing. R Foundation for
608   Statistical Computing, Vienna, Austria, 2020.

609 54. Hothorn T, Bretz F, Westfall P. Simultaneous inference in general parametric models.
610   Biometrical Journal, 2008; 50:346–363.

611 55. Therneau T. A package for survival analysis in R, 2015; R package ver 2.7.

612 56. Mangiafico S, Mangiafico MS. Package 'rcompanion'. Cran Repos, 2017; 20:1–71.

613 57. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D et al. vegan:
614   Community Ecology Package. Ordination methods, diversity analysis and other functions
615   for community and vegetation ecologists, 2018; R package ver 2.5-7.

616 58. Salazar G. EcolUtils: Utilities for community ecology analysis, 2015; R package ver 0.1.

617 59. Loureiro S, Soares AMVM, Nogueira AJA. Terrestrial avoidance behaviour tests as
618   screening tool to assess soil contamination. Environmental Pollution, 2005; 138:121–131.

619 60. Madžarić S, Kos M, Drobne D, Hočevar M, Jemec Kokalj A. Integration of behavioral tests
620   and biochemical biomarkers of terrestrial isopod Porcellio scaber (Isopoda, Crustacea) is
621   a promising methodology for testing environmental safety of chars. Environ Pollut, 2018;
622   234:804–811.

623 61. Tourinho PS, van Gestel CAM, Jurkschat K, Soares AMVM, Loureiro S. Effects of soil and
624   dietary exposures to Ag nanoparticles and $AgNO_3$ in the terrestrial isopod Porcellionides
625   pruinosus. Environ Pollut, 2015; 205:170–177.

626 62. Zidar P, Kos M, Ilič E, Marolt G, Drobne D, Jemec Kokalj A. Avoidance behaviour of
627   isopods (Porcellio scaber) exposed to food or soil contaminated with Ag- and CeO2-
628   nanoparticles. Appl Soil Ecol, 2019; 141:69–78.

629 63. Žižek S, Zidar P. Toxicity of the ionophore antibiotic lasalocid to soil-dwelling
630   invertebrates: Avoidance tests in comparison to classic sublethal tests. Chemosphere,
631   2013; 92:570–575.

632 64. Belbella A, Vauthier C, Fessi H, Devissaguet J-P, Puisieux F. In vitro degradation of
633   nanospheres from poly(D,L-lactides) of different molecular weights and polydispersities.
634   Int J Pharm, 1996; 129:95–102.

635 65. Lyu S, Untereker D. Degradability of polymers for implantable biomedical devices. Int J
636   Mol Sci, 2009; 10:4033–4065.

637 66. deJong SJ, Arias ER, Rijkers D, van Nostrum CF, Kettenes-van den Bosch JJ, Hennink
638   WE. New insights into the hydrolytic degradation of poly(lactic acid): Participation of the
639   alcohol terminus. Polymer, 2001; 42:2795–2802.

640 67. Lunt J. Large-scale production, properties and commercial applications of polylactic acid
641   polymers. Polym Degrad Stabil, 1998; 59:145–152.

642 68. Sander M. Biodegradation of polymeric mulch films in agricultural soils: Concepts,
643   knowledge gaps, and future research directions. Environ Sci Technol, 2019; 53:2304–
644   2315.

645 69. Hajighasemi M, Nocek BP, Tchigvintsev A, Brown G, Flick R, Xu X et al. Biochemical and
646   structural insights into enzymatic depolymerization of polylactic acid and other polyesters
647   by microbial carboxylesterases. Biomacromolecules, 2016; 17:2027–2039.

648   70. Zaaba NF, Jaafar M. A review on degradation mechanisms of polylactic acid: Hydrolytic,
649   photodegradative, microbial, and enzymatic degradation. Polym Eng Sci, 2020; 60:2061–
650   2075.

651   71. Skowron PM, Krefft D, Brodzik R, Kasperkiewicz P, Drag M, Koller K-P. An alternative for
652   proteinase K-heat-sensitive protease from fungus Onygena corvina for biotechnology:
653   cloning, engineering, expression, characterization and special application for protein
654   sequencing. Microb Cell Fact, 2020; 19:135.

655   72. Ohnishi A, Hasegawa Y, Abe S, Bando Y, Fujimoto N, Suzuki M. Hydrogen fermentation
656   using lactate as the sole carbon source: Solution for 'blind spots' in biofuel production.
657   RSC Adv, 2012; 2:8332.

658   73. Seeliger S, Janssen PH, Schink B. Energetics and kinetics of lactate fermentation to
659   acetate and propionate via methylmalonyl-CoA or acrylyl-CoA. FEMS Microbiol Lett,
660   2002; 211:65–70.

661   74. Butbunchu N, Pathom-Aree W. Actinobacteria as promising candidate for polylactic acid
662   type bioplastic degradation. Front Microbiol, 2019; 10:2834.

663   75. Hoshino A, Isono Y. Degradation of aliphatic polyester films by commercially available
664   lipases with special reference to rapid and complete degradation of poly(L-lactide) film by
665   lipase PL derived from Alcaligenes sp. Biodegradation, 2002; 13:141–147.

666   76. Oda Y, Oida N, Urakami T, Tonomura K. Polycaprolactone depolymerase produced by
667   the bacterium Alcaligenes faecalis. FEMS Microbiol Lett, 1997; 152:339–343.

668   77. Kim S-J, Weon H-Y, Kim Y-S, Yoo S-H, Kim B-Y, Anandham R et al. Parapedobacter
669   luteus sp. nov. and Parapedobacter composti sp. nov., isolated from cotton waste
670   compost. Int J Syst Evol Microbiol, 2010; 60:1849–1853.

671   78. Meyer-Cifuentes IE, Werner J, Jehmlich N, Will SE, Neumann-Schaal M, Öztürk B.
672   Synergistic biodegradation of aromatic-aliphatic copolyester plastic by a marine microbial
673   consortium. Nat Commun, 2020; 11:5790.

674   79. Qiu Y-L, Kuang X-Z, Shi X-S, Yuan X-Z, Guo R-B. Terrimicrobium sacchariphilum gen.
675   nov., sp. nov., an anaerobic bacterium of the class 'Spartobacteria' in the phylum
676   Verrucomicrobia, isolated from a rice paddy field. Int J Syst Evol Microbiol, 2014; 64:1718–
677   1723.

678   80. Li C, Cui Q, Li Y, Zhang K, Lu X, Zhang Y. Effect of LDPE and biodegradable PBAT
679   primary microplastics on bacterial community after four months of soil incubation. Journal
680   of Hazardous Materials, 2022; 429:128353.

681   81. Baker JL. Complete genomes of clade G6 Saccharibacteria suggest a divergent
682   ecological niche and lifestyle. mSphere, 2021; 6:e0053021.

683   82. Figueroa-Gonzalez PA, Bornemann TLV, Adam PS, Plewka J, Révész F, Hagen CA von
684   et al. Saccharibacteria as organic carbon sinks in hydrocarbon-fueled communities. Front
685   Microbiol, 2020; 11:587782.

686   83. Yagi H, Ninomiya F, Funabashi M, Kunioka M. Mesophilic anaerobic biodegradation test
687   and analysis of eubacteria and archaea involved in anaerobic biodegradation of four
688   specified biodegradable polyesters. Polym Degrad Stabil, 2014; 110:278–283.

689   84. Hedlund BP, Gosink JJ, Staley JT. Verrucomicrobia div. nov., a new division of the
690   Bacteria containing three new species of Prosthecobacter. Antonie Van Leeuwenhoek,
691   1997; 72:29–38.

692   85. Imhoff JF, Hiraishi A, Sülling J. Anoxygenic phototrophic purple bacteria. In: Brenner DJ,
693   Krieg NR, Staley JR et al. (eds). Bergey's Manual of Systematic Bacteriology: Volume
694   Two: The Proteobacteria, Part A Introductory Essays. Springer, New York, 2005. pp 119–
695   132.

696   86. Kämpfer P, Lodders N, Falsen E. Hydrotalea flava gen. nov., sp. nov., a new member of
697   the phylum Bacteroidetes and allocation of the genera Chitinophaga, Sediminibacterium,
698   Lacibacter, Flavihumibacter, Flavisolibacter, Niabella, Niastella, Segetibacter,
699   Parasegetibacter, Terrimonas, Ferruginibacter, Filimonas and Hydrotalea to the family
700   Chitinophagaceae fam. nov. Int J Syst Evol Microbiol, 2011; 61:518–523.

701   87. McBride MJ, Liu W, Lu X, Zhu Y, Zhang W. The Family Cytophagaceae. In: Rosenberg
702   E., DeLong E.F., Lory S. et al. (eds). The Prokaryotes. Springer, Berlin, 2014. pp 577–
703   593.

704   88. Wu Y-F, Wu Q-L, Liu S-J. Chryseobacterium taihuense sp. nov., isolated from a eutrophic
705   lake, and emended descriptions of the genus Chryseobacterium, Chryseobacterium
706   taiwanense, Chryseobacterium jejuense and Chryseobacterium indoltheticum. Int J Syst
707   Evol Microbiol, 2013; 63:913–919.

708 89. Yoon J-H, Kang S-J, Park S, Oh T-K. Devosia insulae sp. nov., isolated from soil, and
709   emended description of the genus Devosia. Int J Syst Evol Microbiol, 2007; 57:1310–
710   1314.

711 90. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR et al. Enterotypes
712   of the human gut microbiome. Nature, 2011; 473:174–180.

713 91. Yun J-H, Roh SW, Whon TW, Jung M-J, Kim M-S, Park D-S et al. Insect gut bacterial
714   diversity determined by environmental habitat, diet, developmental stage, and phylogeny
715   of host. Appl Environ Microbiol, 2014; 80:5254–5264.

716 92. Clegg CD, van Elsas JD, Anderson JM, Lappin-Scott HM. Assessment of the role of a
717   terrestrial isopod in the survival of a genetically modified pseudomonad and its detection
718   using the polymerase chain reaction. FEMS Microbiol Ecol, 1994; 15:161–168.

719 93. Clark DP. The fermentation pathways of Escherichia coli. FEMS Microbiol Lett, 1989;
720   63:223–234.

721 94. Osborne CA, Peoples MB, Janssen PH. Detection of a reproducible, single-member shift
722   in soil bacterial communities exposed to low levels of hydrogen. Appl Environ Microbiol,
723   2010; 76:1471–1479.

724 95. Schlesinger WH. Biogeochemistry 2nd Ed. Academic Press, San Diego, California, 1997.

725 96. Koyama T. Gaseous metabolism in lake sediments and paddy soils and the production of
726   atmospheric methane and hydrogen. J Geophys Res, 1963; 68:3971–3973.

Figure Legends

Fig. 1: Representative radial hydrogen profiles of isopod guts. The isopods were fed with food containing no microplastic particles (control; **a**) or 5% PLA (**b**), PET (**c**) or PS (**d**) for 6 days prior gut extraction and subsequent embedding in agarose and microsensor measurements. For each gut, profiles were recorded from the anterior, median and posterior. Analyses of two more guts per treatment are displayed in Fig. S6. Closed and open symbols represent measured concentrations inside and outside (in agarose) the guts, respectively. The distance of 0 µm indicates the center of the tube-like gut.

Fig. 2: Hydrogen accumulation in the headspace of whole isopods incubated under air for 10 h. Values represent means of triplicate vials each containing three isopods that were analyzed directly after collection and not subjected to a MP-exposure experiment. The dot-dashed line indicates a linear regression of the hydrogen mixing ratios. Means and standard errors of three replicates are plotted. The regression equation including the standard error of the slope is shown near the regression line.

Fig. 3: Effects of MP ingestion on the abundance of bacterial 16S rRNA genes and 16S rRNA in the gut and respective food of *P. scaber.* Nucleic acid extracts derived from the guts of isopods (**a, b, c**) that were exposed to control- or 2.5%-MP- (striped) or 5%-MP- (no pattern) food pellets (**d, e, f**) were directly used for quantification of genes (**a, d**). A subsample of each extract was subjected to DNase treatment and subsequent reverse transcription for analysis of 16S rRNA (**b, e**). In addition, the 16SrRNA:16S rRNA gene ratios were calculated (**c, f**). The data was corrected for the proportion on endosymbionts obtained from sequencing analysis (see supplementary Fig. S7 for comparison of uncorrected and corrected data). Means and standard deviations of five replicates are plotted. Statistical analysis revealed no effect of the concentration of MP applied and therefore, significant differences in means indicated by different lower letters above the bars are related the MP treatment regardless the dosage.

753 **Fig. 4: Beta diversity of the active bacterial gut communities.** PCoA plots are based on

754 Aitchison distance matrixes derived from analyses of the 16S rRNA genes and 16S rRNA.

755 Results of the PERMANOVA analyses are given for each plot. Arrows represent ASVs

756 assigned on family and genus/species level (if applicable) that were highly correlated with the

757 separation of samples.

758 **Fig. 5: Shared and unique numbers and proportions of taxa among guts of isopods fed**

759 **with control-, PLA-, PET- and PS-food on 16S rRNA level.** Only taxa on genus level (if

760 applicable) that occur in at least 30% of the replicates were included for the calculation of the

761 Venn diagram. The scale indicates the count numbers of taxa in correlation with the intensity

762 of the shading.

763 **Fig. 6: Indicator taxa in isopod guts on 16S rRNA level.** The relative abundances of taxa

764 were normalized with the total 16S rRNA abundance derived from qPCR analysis. Indicator

765 taxa for the PLA- (and control-) (**a**) or PET- (and control-) (**b**) treatments were identified. Only

766 taxa on genus level (if applicable, otherwise lowest classification and the number of ASVs are

767 given) that occur in at least three replicates were accepted as potential indicators.

768 **Fig. 7: Potential mixed acid fermentation pathway by *Enterobacteriaceae* initiated with**

769 **lactate.** Under conditions, at which pyruvate generated from glucose (or similar compounds)

770 is limited and lactate (that could be derived from PLA as indicated by a dotted arrow) is

771 available, two lactate are oxidized by lactate dehydrogenase (LDH) yielding two pyruvate and

772 two NADH. Two pyruvate react to two acetyl-CoA and two formate catalysed by pyruvate

773 formate lyase (PFL). One acetyl-CoA is then converted to acetate via phosphotransacetylase

774 (PTA) and acetate kinase (AK) yielding one ATP, the other acetyl-CoA is reduced to ethanol

775 by alcohol dehydrogenase (ADH) thereby using two NADH. Formate hydrogen lyase (FHL)

776 transforms formate to carbon dioxide and molecular hydrogen preferentially under acidic

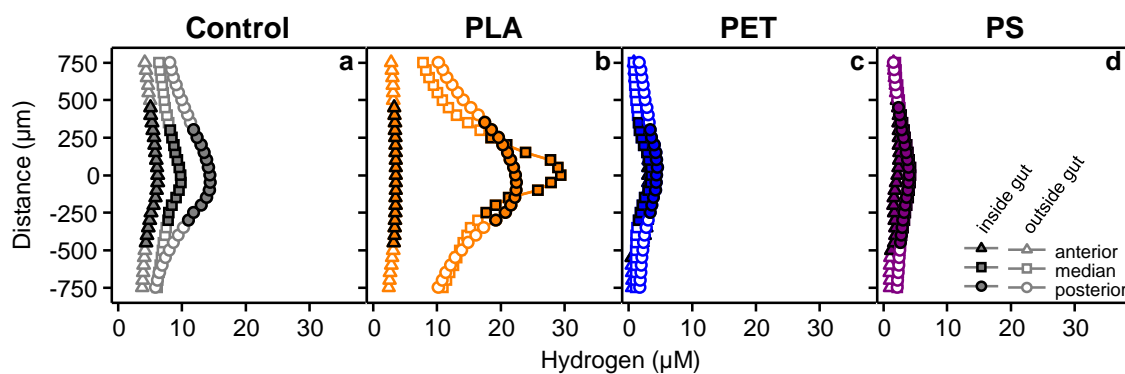777 conditions (indicated by a dashed arrow).

Fig. 1

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Fig. 2

y = (0.83 ± 0.51) x

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Fig. 3

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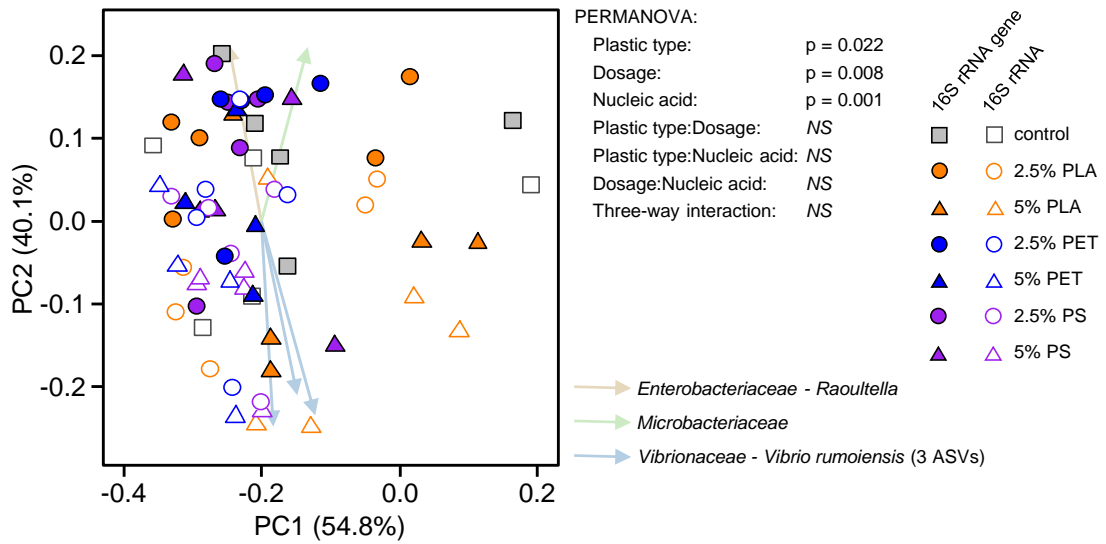
Fig. 4

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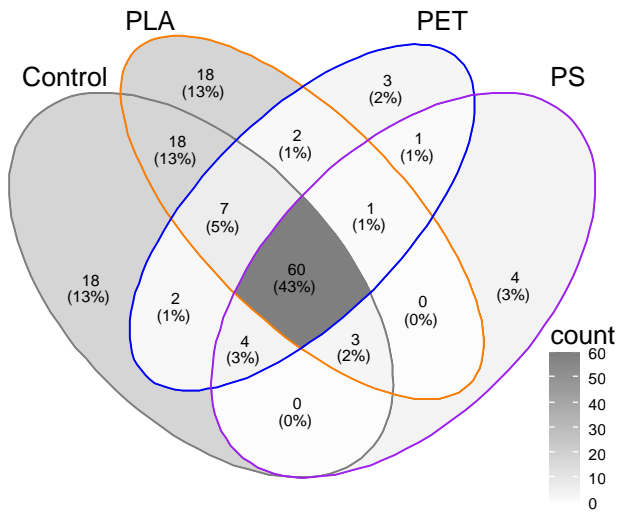
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Fig. 5: Shared and unique numbers and proportions of taxa among guts of isopods fed with control-, PLA-, PET- and PS-food on 16S rRNA level. Only taxa on genus level (if applicable) that occur in at least 30% of the replicates were included for the calculation of the Venn diagram. The scale indicates the count numbers of taxa in correlation with the intensity of the shading.
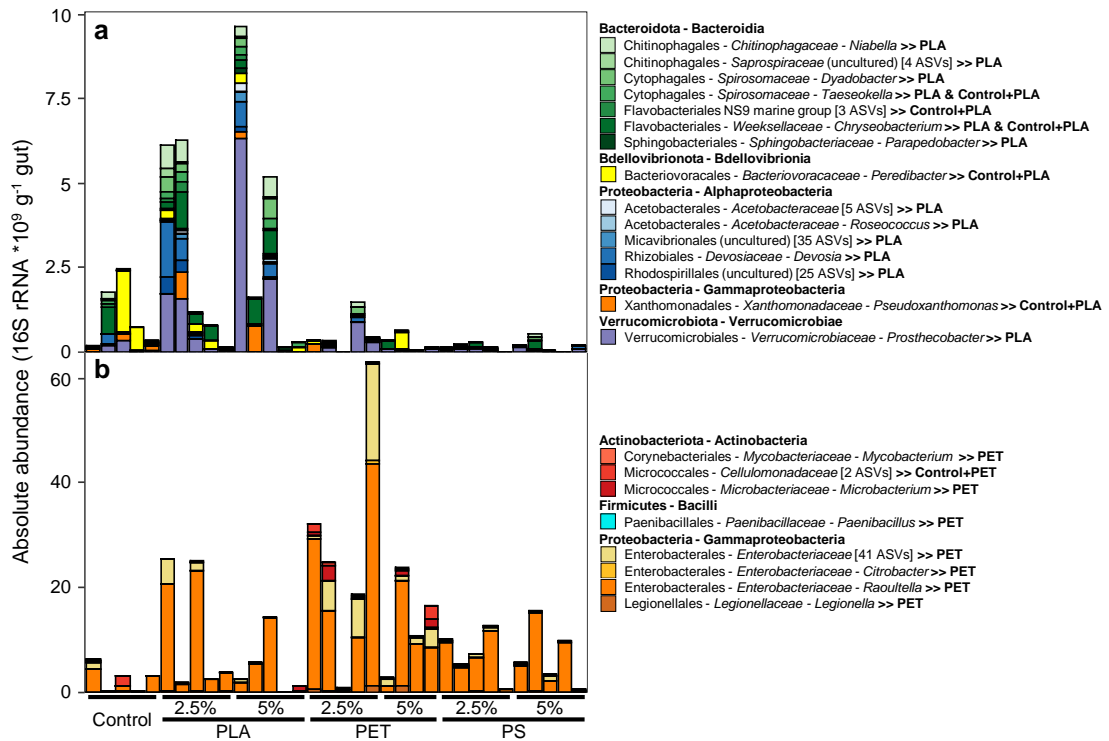
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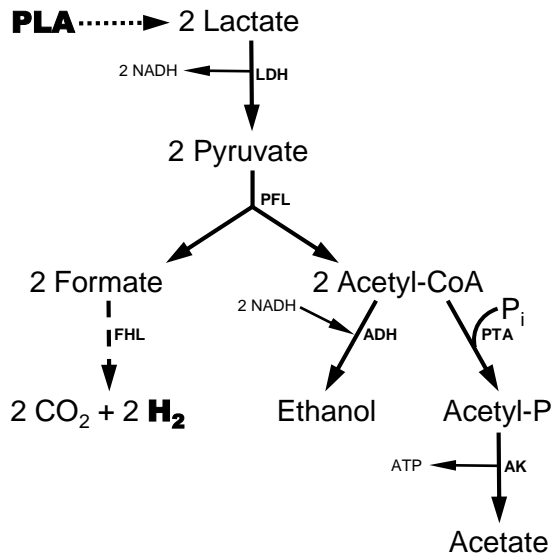
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