Received: 8 September 2022 | Accepted: 31 March 2023

DOI: 10.1111/1462-2920.16386

RESEARCH ARTICLE

ENVIRONMENTAL MICROBIOLOGY Applied Microbiology International

Microplastic ingestion affects hydrogen production and microbiomes in the gut of the terrestrial isopod *Porcellio scaber*

Linda Hink[1] | **Anja Holzinger**[2] | **Tobias Sandfeld**[3] | **Alfons R. Weig**[4] | **Andreas Schramm**[3] | **Heike Feldhaar**[2] | **Marcus A. Horn**[1]

[1]Institute of Microbiology, Leibniz University Hannover, Hannover, Germany

[2]Animal Population Ecology, Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, Bayreuth, Germany

[3]Department of Biology, Section for Microbiology, Aarhus University, Aarhus, Denmark

[4]Genomics and Bioinformatics, University of Bayreuth, Bayreuth, Germany

Correspondence
Marcus A. Horn, Institute of Microbiology, Leibniz University Hannover, Herrenhäuserstr. 2, 30419 Hannover, Germany.
Email: horn@ifmb.uni-hannover.de

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: 391977956

Abstract

Microplastic (MP) is an environmental burden and enters food webs via ingestion by macrofauna, including isopods (*Porcellio scaber*) in terrestrial ecosystems. Isopods represent ubiquitously abundant, ecologically important detritivores. However, MP-polymer specific effects on the host and its gut microbiota are unknown. We tested the hypothesis that biodegradable (polylactic acid [PLA]) and non-biodegradable (polyethylene terephthalate [PET]; polystyrene [PS]) MPs have contrasting effects on *P. scaber* mediated by changes of the gut microbiota. The isopod fitness after an 8-week MP-exposure was generally unaffected, although the isopods showed avoidance behaviour to PS-food. MP-polymer specific effects on gut microbes were detected, including a stimulation of microbial activity by PLA compared with MP-free controls. PLA stimulated hydrogen emission from isopod guts, while PET and PS were inhibitory. We roughly estimated 10^7 kg year^{-1} hydrogen emitted from the isopods globally and identified their guts as anoxic, significant mobile sources of reductant for soil microbes despite the absence of classical obligate anaerobes, likely due to *Enterobacteriaceae*-related fermentation activities that were stimulated by lactate generated during PLA-degradation. The findings suggest negative effects of PET and PS on gut fermentation, modulation of important isopod hydrogen emissions by MP pollution and the potential of MP to affect terrestrial food webs.

INTRODUCTION

Today's modern life without plastic is inconceivable, as plastic is highly versatile and can be applied in various sectors ranging from the packaging to the building and construction sector with a likewise wide range of lifetimes from less than a year to several decades. To date more than 8.3 billion tonnes have been produced, out of which about 60% have been discarded. A large proportion of the plastic waste is either disposed of in landfills or ends as litter in the natural environment (Geyer et al., 2017). Hence, plastic is not only advantageous but also has become an ubiquitous man-made environmental burden. Due to weathering and fragmentation, large plastic items are gradually transformed to slowly degrading microplastic (MP) smaller than 5 mm in diameter (Andrady, 2017; Hartmann et al., 2019). Although biodegradation by specialized microorganisms originating from plastic polluted sites, landfills or animal intestines has been observed (Gambarini et al., 2021), MPs only degrade slowly in natural environments and persist over decades (Lebreton et al., 2019). While most attention so far focused on oceanic environments, the annual plastic release to

terrestrial environments is 4–23 times higher, mostly due to agricultural practices and littering (Horton et al., 2017). Conventional plastics, such as polyethylene (PE), polyethylene terephthalate (PET), polystyrene (PS) and polyvinyl chloride (PVC), are considered to be extremely resistant to biodegradation. Biodegradable polymers such as polylactic acid (PLA) and polycaprolactone are therefore becoming popular as an alternative. However, their degradation is incomplete and slow under environmental conditions (Emadian et al., 2017).

MP may not only alter the physical and chemical soil properties, but may also affect the soil biota (Büks et al., 2020; Büks & Kaupenjohann, 2022; Ji et al., 2021). Unintentional ingestion of MP by soil-dwelling and especially soil-feeding macrofauna is likely and can have negative fitness effects on organisms like earthworms, nematodes or collembola (Büks et al., 2020; Ji et al., 2021). Subsequently, MP may be transferred to higher trophic levels, as shown for chickens that acquired MP from soil by feeding on earthworms (Huerta Lwanga et al., 2017).

Like earthworms, also terrestrial isopods (i.e., woodlice—Isopoda—Oniscidea) are widespread decomposers with a density that can exceed 1000 individuals m^2 (Paoletti & Hassall, 1999). These organisms are sensitive to contaminants, for example, pesticides or heavy metals, and thus are suited for soil ecotoxicity testing in laboratory and field bioindicator studies (Paoletti & Hassall, 1999; van Gestel et al., 2018). Isopods are known to be sensitive to evaporation and thus prefer moist habitats, which in turn often comes along with darkness, and they thus dwell in the upper soil and litter layers (Edney, 1954). As soil-dwellers, they mainly feed on decaying leaf litter and wood, and it has been shown that weathered feed colonized by microbes is actually favoured (Hassall et al., 1987; Ihnen & Zimmer, 2008). Along with their preferred food sources, isopods also ingest soil (Zimmer, 2002). Their presence in soil enhances soil nutrient cycling due to the fragmentation and transportation of organic material along with microorganisms (Rabatin & Stinner, 1988). Hence, it can be considered that they likewise contribute to the fragmentation and transportation of accidently ingested MP and even distribution of pathogenic microorganisms as plastic surfaces are suitable for colonization (Gkoutselis et al., 2021; Kirstein et al., 2016; Rohrbach et al., 2022). Many studies have investigated the effects of pollutants on the isopod *Porcellio scaber* (common rough woodlouse; van Gestel et al., 2018). However, to date there are only a few studies investigating life history traits after MP ingestion: no or only minor effects on survival, feeding rate, body mass or energy reserves in the digestive glands were obtained for isopods exposed to PE particles, tire particles or polyester fibres (Jemec Kokalj et al., 2018; Selonen et al., 2020, 2021). However, this does not necessarily mean that *P. scaber* is only marginally affected by MP ingestion. The effects are possibly less obvious after a relatively short exposure of only a few weeks and the detection of sublethal effects requires assessment of other parameters. Accordingly, immune response parameters are affected by MP (polyester fibres and tire particles) ingestion (Dolar et al., 2021, 2022). Such effects are potentially linked to effects on the gut microbiota, but the effects of MP exposure on the gut microbiota of *P. scaber* have not been investigated yet. In case of MP effects on the gut microbiota, potential consequences are alteration of the metabolites produced through microbial activity that may not only affect the host. Such altered gut communities and their metabolites are likely to be released into the soil ecosystem. This in turn may have profound downstream effects like environmental niche alterations and changes of the soil microbiota.

Generally, an intact gut microbiota is important for the development, nutrition and immunity and this also applies for isopods that possess a more dynamic microbiota compared with higher organisms, such as mammals (Bouchon et al., 2016). The nutrient content in the common diet of isopods (leaf litter and dead wood) is generally very low and it is suggested that isopods rely on microbes colonizing and degrading the decaying plant material and thereby providing nutrients for the host, or the microbes themselves are digested and serve as a nutrient-rich source (Horváthová et al., 2016; Ihnen & Zimmer, 2008; Zimmer, 2002). With a reduced microbial cell number in the anterior section of the hindgut, it is likely that the latter applies for this gut section, while microbes proliferate towards the posterior section, where mainly anaerobic conditions prevail (Drobne, 1995; Zimmer & Brune, 2005; Zimmer & Topp, 1998). The most abundant groups in *P. scaber's* gut microbiota have been assigned to Proteobacteria, Bacteroidota and Actinobacteria commonly inhabiting insect intestines (Horváthová et al., 2016, 2019; Kostanjsek et al., 2002, 2004). All of these phyla contain members that possess a facultative or even obligate anaerobic lifestyle and therefore, fermentative microbes including hydrogen producers may play an important role as shown for other invertebrates (e.g., termites and earthworms; Ebert & Brune, 1997; Wüst et al., 2009). However, whether or not fermentation is an ongoing process in the gut of *P. scaber* remains to be determined.

The gut microbial community and hence the digestive processes can indeed be modulated by MP ingestion as shown for several soil invertebrates: In the gut of mealworms (*Tenebrio molitor* larvae), PE and PS can be degraded with a strong association of species within the *Enterobacteriaceae* (Brandon et al., 2018). Adverse effects of MP ingestion on life history traits (e.g., reduced growth and reproduction rates) appear along with alteration of the gut microbiota of springtails

(*Folsomia candida*; ingestion of PVC or PE; Zhu, Chen, et al., 2018; Ju et al., 2019) and potworms (*Enchytraeus crypticus*; ingestion of PS; Zhu, Fang, et al., 2018). The effects of biodegradable MP on invertebrate gut microbiota have not been investigated yet, and generally studies of *P. scaber's* gut microbiota after any MP ingestion are lacking to date.

This study aims to investigate the effects of conventional non-biodegradable MP particles, PET and PS, and biodegradable PLA on *P. scaber* with respect to fitness, gut microbiota and fermentation potential in the gut, with the underlying hypothesis that biodegradable and non-biodegradable MPs have contrasting effects on *P. scaber* mediated by changes of the associated gut microbiota. This was tested in MP-feeding experiments, microsensor analyses of prevailing conditions in the gut with respect to pH, oxygen and hydrogen (as a measure for ongoing microbial fermentation) concentrations and analyses of the gut microbiota. In addition, the food microbiota was analysed to investigate its influence on the gut microbiota.

EXPERIMENTAL PROCEDURES

Food preparation and isopod collection

Food pellets consisting of withered leaves (mainly maple leaves; 42%), ground commercial rabbit food (25%) and potato powder (33%) were prepared as described in Zižek et al. (2011). For the pellets that additionally contained MP fragments, PLA, PET and PS granules were purchased from NatureWorks (Naarden, The Netherlands), Veolia (Berlin, Germany) and Ineos Styrolution (Ludwigshafen, Germany), respectively. MP was produced from granules by grinding to fragments using a cryo ball mill (Retsch, CryoMill, Germany) and followed by sieving to obtain fragments ranging from 75 to 150 μm in diameter of irregular shape. Then, 2.5% or 5% (w/w) PLA, PET or PS was added to the food mixture.

P. scaber individuals (only adults; weight >30 mg) as model isopods were collected in a garden near the campus of the University of Bayreuth (Germany) or the Leibniz University of Hannover (Germany) between February and May 2020. Reproducible behaviour of isopods collected from two sampling sites was expected, as habitats, food sources and temperate climatic conditions were similar. The animals were kept in boxes (40 × 30 × 25 cm) filled with damp soil, leaves and tree bark prior to the performance of independent experiments assessing hydrogen and methane emission rates of whole isopods, microsensor profiles of pH, hydrogen and oxygen concentrations of isopod guts, bacterial community composition of the isopod guts and food pellets, as well as fitness effects and food choice (for the latter see Supplementary Experimental Procedures).

Molecular hydrogen, oxygen and pH microsensor measurements from isopod guts

Microsensor measurements were performed to identify the location and level of hydrogen production within isopods as an estimate for the fermentation potential and to assess MP effects on the conditions inside the gut of the isopods. One gram of food pellets containing no MP or 5% PLA, PET or PS were mixed with 2 mL 1% agar (≈60°C), spread on a petri dish and cooled to room temperature. Twelve isopods per treatment were placed on these petri dishes and kept at room temperature in the dark. The food was exchanged after 3 days and isopods were kept for three further days. Prior to gut dissection, the isopods were placed on ice for several minutes in order to lower their mobility. Each gut was embedded within a small glass chamber in 1% low-melt agarose in insect Ringer's solution. Coverslips and microscope slides (7.5 × 2.5 × 0.1 cm) were used for the construction of chambers similar to those in Brune et al. (1995): a coverslip at the bottom of the chamber and two microscope slides on top of each other were arranged to each side of the chamber providing the dimensions of 2.5-cm length, 1.0-cm width and 0.2-cm depth. The bottom of the chamber was filled with a layer of molten 1% low-melt agarose in insect Ringer's solution and after solidification, a freshly dissected full isopod gut was placed on top of it. Then a top agarose layer (not warmer than 40°C) was cast in the chamber, which was immediately covered with a coverslip before solidification. The agarose block with a single gut embedded was placed on another 2-mm thick agarose bed in a weighing boat, which was used to prevent microsensor destruction in case the tip went too low, that is, beyond the agarose block. Insect Ringer's solution just covering the upper surface of the agarose block was applied to prevent dehydration of the agarose blocks and thus provide similar conditions for multiple microsensor measurements that were performed for a single gut.

Custom-made microsensors for oxygen, hydrogen and pH (Ebert & Brune, 1997; Revsbech, 1989; Revsbech & Jørgensen, 1986; Schramm, 2006) with tip diameters <20 μm were used for recording radial profiles of the anterior (at a distance of 1 mm from the front end), the medial and the posterior (at a distance of 1 mm from the rear end) of isopod guts (see positions in Figure S1). Measurements were performed at room temperature. For pH measurements, a bridge consisting of a syringe barrel filled with the same agarose as the agarose bed was constructed between this bed and

using an Ag/AgCl reference electrode with Red Rod technology (Radiometer REF201). The sensors were connected to a four-channel multimeter with a built-in 16-bit A/D converter (Unisense Microsensor Multimeter, Ver 2.01; Unisense A/S, Denmark). Pre-polarized sensors were calibrated prior to gut profile measurements: a two-point calibration with a 0.7 M alkaline ascorbate solution (0 μM oxygen) and an air-saturated Ringer's solution (265.6 μM oxygen) for the oxygen microsensor; a three-point calibration with pH 4, 7 and 10 buffers for the pH microsensor; a calibration with multiple points ranging between 0 and 50 μM for the hydrogen microsensor. Data acquisition and control of the microprofiling system were enabled with the software program SensorTrace PRO (Unisense A/S). Profiling through the guts was performed with 50 μm spatial resolution. Oxygen and pH sensors were allowed to equilibrate for 5 s prior to data acquisition. For hydrogen sensors, 10 s equilibration time were required. The measurements with the different sensors were performed with different guts. Three guts per treatment and microsensor were analysed (in total, 9 of 12 original specimens from the petri dish) at three positions each (anterior, medial and posterior).

Determination of molecular hydrogen and methane emission from whole isopods

In order to confirm hydrogen emissions under in vivo conditions, hydrogen production rates of whole isopods were determined. In addition to hydrogen, methane is relevant in the anaerobic food chain and was also analysed. Therefore, adult isopods were freshly collected and surface sterilized with 70% ethanol. These animals were not subjected to a MP-treatment. Groups of three individuals each were placed in three 3-mL Exetainer (Labco, Lampeter, UK). The vials were sealed with airtight lids (caps with butyl septa) and overpressure was applied to all vials via injection of 2 mL air. Headspace hydrogen and methane mixing ratios were analysed for a period of 10 h with a gas chromatograph coupled to a pulsed discharge helium ionization detector (7890B, Agilent Technologies, Santa Clara, CA, USA) via a micro-packed molecular sieve column (ShinCarbon ST 80/100, 2 m, 0.5 mm ID, Part#: 19043, Restek Corporation, Bellefonte, PA, USA) and a capillary column (RT-Molecular Sieve 5A, 30 m, 0.53 mm ID, 50 μm, Cat#: 19723, Restek Corporation) as described in Zaman et al. (2021).

Isopod feeding experiment with assessment of fitness parameters

Groups of 10 isopods (7 females, 3 males) were kept in glass jars (diameter: 10.8 cm; volume: 370 mL) with the bottom covered with moist filter paper in a climate chamber with a 16 h light and 8 h dark cycle at 16°C and 85% humidity. Isopod groups were exposed to food pellets without or with 2.5% or 5% PLA, PET or PS for 8 weeks. Each treatment was performed in five replicates (five glass jars per treatment containing 10 isopods each). One food pellet was placed in each jar and exchanged every other day. At the same time, the survival of isopods was checked and dead individuals were removed. Once a week, the glass jars were cleaned and the filter papers were replaced. Locomotor activity tests were performed after 2, 4 and 6 weeks (see Supplementary Experimental Procedures). After 8 weeks, the isopods were weighed and the percentage weight gain to the initial weight was calculated. Furthermore, guts were dissected from one specimen per jar and these guts as well as the food pellet of the respective treatment (one pellet per jar) were frozen in liquid nitrogen and kept at −80°C prior to microbiota analysis.

Nucleic acid extraction, DNase treatment and reverse transcription

Prior to extraction of DNA and RNA from one whole isopod gut per replicate (jar), their weight was determined. Five extractions per treatment yielding 35 nucleic acid extractions in total were performed. The nucleic acids were also extracted from subsamples of the food pellets (≈50 mg; likewise 5 extractions per treatment yielding 35 extractions in total). The extraction protocol was performed according to Griffiths et al. (2000) with some modifications: (i) 2-mL screw cap tubes used during initial cell lysis were filled with Ø0.1-mm and Ø0.5-mm zirconium beads, 150 mg each, and one Ø3-mm glass bead; (ii) cell lysis by bead beating for 30 s at 5.0 m s^{-1} was performed twice with an intermitted cooling on ice for 30 s; (iii) nucleic acids were precipitated on ice for 2 h; (iv) air-dried nucleic acid pellets derived from gut and food samples were resuspended in 30 and 60 μL RNase-free water, respectively. Verification of nucleic acid extracts was assessed via agarose gel electrophoresis and spectrophotometric measurements. DNase treatment was applied to a 13-μL subsample of each extract using the TURBO DNA-free™ Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Reverse transcription of 10 μL RNA was performed using LunaScript RT Supermix Kit (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's protocol. Negative controls without template RNA (water treated with DNase) and, for each sample, without reverse transcriptase were performed. Samples were kept on ice for further processing.

Analysis of bacterial 16S rRNA genes and 16S rRNA

Bacterial 16S rRNA genes and 16S rRNA (after reverse transcription) were quantified by quantitative polymerase chain reaction (qPCR) using primers Bact_341F and Bact_805R (Herlemann et al., 2011). Each sample was analysed in duplicate 10-μL reactions containing 5 μL Luna Universal qPCR Master Mix (New England Biolabs), 2 μM of each primer, 10 μg bovine serum albumin and 2 μL of 1:100 diluted template (c)DNA. Negative controls contained sterile water instead of template (c)DNA. Standards consisted of serially diluted (10^2–10^6 gene copies per μL) M13uni/rev PCR products of a pGEM-T vector with a 16S rRNA gene inserted. The amplification was performed in a CFX Connect Real-Time PCR System (Bio-Rad, Feldkirchen, Germany) with the following cycling conditions: 2 min at 95°C, 35 cycles of 30 s at 95°C, 50 s at 60°C (combined annealing and elongation) and a plate read after 10 s at 80°C. The amplification efficiencies ranged between 96% and 98%, the r^2 values were ≥0.99. The specificity of the amplification was verified by melting curve analysis (from 60 - 95°C in 0.5°C intervals for 5 s each) and agarose gel electrophoresis. Specific amplification of archaeal 16S rRNA (using primers A519F; Wang & Qian, 2009 and A1017R; Yoshida et al., 2005) and [Fe-Fe]-hydrogenase genes (encoding for enzymes that catalyse the production of hydrogen in obligate anaerobic fermenting bacteria, using primers from Schmidt et al. (2010) and Xing et al. (2008)) was tested for several gut and food samples, but consistently failed.

Bacterial 16S rRNA genes and 16S rRNA were also analysed by high-throughput amplicon sequencing. Amplicons were generated using the same primer pairs as for the qPCR, but tagged with specific adapters (Illumina, San Diego, USA). Each reaction mixture contained 12.5 μL Kapa HiFi HotStart ReadyMix (Roche, Mannheim, Germany), 0.5 μM of each primer, 5 μg bovine serum albumin and 2.5 μL of 1:10 diluted template (c)DNA. Sterile water, instead of template was applied for negative controls. The PCR was performed in a thermocycler (Biozym Scientific GmbH, Hessisch Oldendorf, Germany) with 3 min initial denaturation at 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-elongation step at 72°C for 1 min. Specificity of the amplification was confirmed by agarose gel electrophoresis. The amplicons were purified using the GeneRead size selection kit (Qiagen, Hilden, Germany). Library preparation was performed using the Nextera XT Index Kit (Illumina) as given elsewhere (Ho et al., 2020) and the Illumina MiSeq version 3 chemistry was applied for 2 × 300 bp paired-end sequencing.

After demultiplexing of the sequencing reads, the QIIME2 software package (version 2021.2; www.qiime2.org) was used for further processing as described previously (Weig et al., 2021). Briefly, primer specific sequences were removed from sequencing reads that were subsequently subjected to the DADA2 pipeline for quality filtering, denoising and joining of paired reads. Low-abundant amplicon sequencing variants (ASVs) with frequencies below the median frequency per ASV of seven were filtered out and the remaining ASVs were taxonomically classified using a trained classifier based on the SILVA database (version 138; www.arb-silva.de). ASVs shorter than 390 bp and those matching 'chloroplast' or 'mitochondria' were removed from the data set. Entirely unclassified ASVs or those assigned to unclassified bacteria were manually inspected for chimera formation via NCBI blastn search (www.blast.ncbi.nlm.nih.gov) and excluded in case of the first, and the second half of the respective reads was attributed to distanced taxa. If not stated otherwise, ASVs classified as the widespread endosymbionts *Wolbachia* or *Candidatus* Hepatincola (Bouchon et al., 2016) likely derived from the gut tissue were also eliminated. The abundance of 16S rRNA genes and 16S rRNA derived from qPCR analyses was recalculated excluding the proportion of these endosymbionts. With the exception of one sample with less than 5000 reads that was discarded (16S rRNA derived from food containing 2.5% PS), all samples contained at least 20,000 reads and were analysed further. Based on the Aitchison distance (a compositional distance metric calculated with the DEICODE plugin; Martino et al., 2019), the beta diversity of the whole data or of a subset (only food samples, only gut samples) was analysed. Visualization of the microbial community data was performed with R (version 4.1.1; R Core Team, 2020) using the packages *phyloseq* (McMurdie & Holmes, 2013) and *ggplot2* (Villanueva & Chen, 2019). Overlapping (shared) or exclusive (unique) taxa (on genus level, if applicable) in multiple (sub-)data sets was demonstrated using Venn diagrams with the R package *ggVennDiagram* (Gao et al., 2021). Only taxa that occurred in at least 30% of the respective replicates were included. An indicator species analysis on genus level (if applicable) with the multipatt function in the *indicspecies* package of R (1000 permutations) was conducted to assess specific associations of taxa with specific origin (isopod gut or food) using the relative of 16S rRNA gene and 16S rRNA abundances (de Cáceres & Legendre, 2009). The analyses of the associations of taxa with a particular MP-treatment were performed on absolute 16S rRNA gene and 16S rRNA abundances using the results of the total quantification (qPCR data).

Statistical analysis

All statistical analyses were conducted using the statistical platform R version 4.1.1 (R Core Team, 2020).

Differences in weight gain, food choice and speed index of the isopods as well as maximum hydrogen concentration and minimum pH in the isopod guts assessed via microsensor profiles were analysed by fitting linear models, performing analysis of variance (ANOVA) tests and Tukey post hoc comparisons using the *multcomp* package (Hothorn et al., 2008). The survival probability of isopods and confidence intervals were calculated using the *survival* package (Therneau, 2015). Abundance data obtained from qPCR analysis were investigated by a factorial two-way ANOVA (MP-treatment and percentage as factors). If the initial model has not met normality assumptions, the data were transformed using the transformTukey function in the *rcompanion* package (Mangiafico & Mangiafico, 2017), and then the adjusted model met the required assumptions. Tukey post hoc tests were applied to assess significant differences in means. Whether the community compositions differed between the treatments was assessed with permutational multivariate ANOVA (PERMANOVA) tests and pairwise comparisons applied on the Aitchison distance matrix using the adonis2 and the adonis.pair function in the *vegan* (Oksanen et al., 2018) and *EcolUtils* (Salazar, 2015) packages of R, respectively.

RESULTS

Effects of MP on isopods

When *P. scaber* had the choice between food containing no MP or 5% PLA, PET or PS, a significant avoidance of PS-food was observed (Figure S2A). However, when there was no choice given, any food was ingested. Neither the survival (≈80% of individuals survived after 8 weeks; Figure S3), nor the weight gain was affected by the MP-diet ($p = 0.556$; Figure S2B). The speed index for isopods exposed to 2.5% PET was significantly lower than that of those exposed to 2.5% PS; nevertheless, none of the MP-food had a significant effect compared to the control-food (Figure S2C).

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

Radial oxygen micro-profiles revealed anoxic conditions at any position in the guts of isopods fed with any diet, and pH ranged from 5 to 7 (Figures S4 and S5). Minimum gut pH values were not affected by the MP-treatment nor an interaction of position and MP-treatment (ANOVA; $p > 0.05$). The minimum pH was significantly more acidic in the anterior (pH 5.2) than in the medial to posterior (pH 5.8) positions (Figure S5, Table S1). Hydrogen was highest in the centre of all isopod guts at all measured positions (Figures 1 and S6). Hydrogen concentrations of up to ≈20 μM were detected in the gut centre of isopods fed with MP-free control food. Isopods fed with PLA-food showed highest and those fed with PET- or PS-food lowest gut hydrogen concentrations. At the gut medial, maximum hydrogen concentrations of ≈30 μM were significantly higher in isopods fed with PLA-food than with other food (Table S2). At the posterior position of the gut, maximum hydrogen concentrations of ≈5 μM were significantly lower in isopods fed with PET- and PS-food than with control- and PLA-food. It is also worth mentioning that in the guts of isopods fed with control- and PLA-food, the hydrogen formation activity was significantly higher towards the posterior than at the anterior end.

Hydrogen emission potential of whole isopods

Hydrogen mixing ratios in the headspace of vials containing whole isopods (analysed directly after collection and not subjected to MP-treatments) increased linearly over time without appreciable delay (Figure 2), demonstrating that hydrogen is indeed emitted from whole isopods under in vivo conditions. The emission rates were highly variable with an average rate of 0.83 ± 0.51 ng hydrogen isopod^{-1} h^{-1} resulting in 1–24 ng hydrogen isopod^{-1} after 10 h of incubation.

Impact of MP on 16S rRNA gene and 16S rRNA abundance in food and guts of isopods

Bacterial 16S rRNA abundances were essentially one order of magnitude higher in the isopod guts than in the food pellets, while the opposite applied for 16S rRNA genes and with this, the 16S rRNA:16S rRNA gene ratios were consistently higher in the guts than in the food despite a high variability among replicates (Figure 3). 16S rRNA gene abundances obtained from the guts were significantly higher in isopods exposed to PLA-food compared to the other treatments (Figure 3A). A similar stimulation was reflected at 16S rRNA level, but the 16S rRNA:16S rRNA gene ratios were unchanged across treatments (Figure 3B,C). MP had no significant effects on the 16S rRNA gene and 16S rRNA abundances in the food pellets and ratios thereof (Figure 3D–F).

Impact of MP on the bacterial communities

The bacterial communities in the isopod guts were highly diverse (Figure S8C,D). Highest relative gene abundances were found for taxa within the

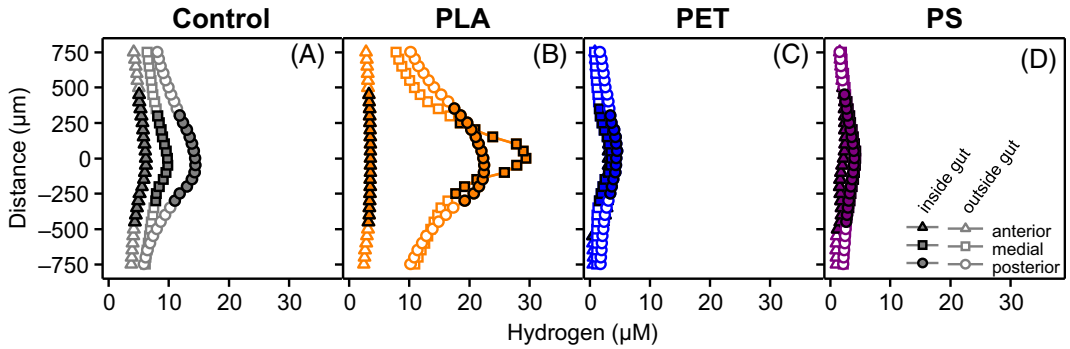

FIGURE 1 Representative radial hydrogen profiles of isopod guts. The isopods were fed with food containing no microplastic particles (control; A) or 5% polylactic acid (PLA) (B), polyethylene terephthalate (PET) (C) or polystyrene (PS) (D) for 6 days prior to gut extraction and subsequent embedding in agarose and microsensor measurements. For each gut, profiles were recorded from the anterior, medial and posterior. Analyses of two more guts per treatment are displayed in Figure S6. Closed and open symbols represent measured concentrations inside and outside (in agarose) the guts, respectively. The distance of 0 µm indicates the centre of the tube-like gut as visualized in Figure S1B.

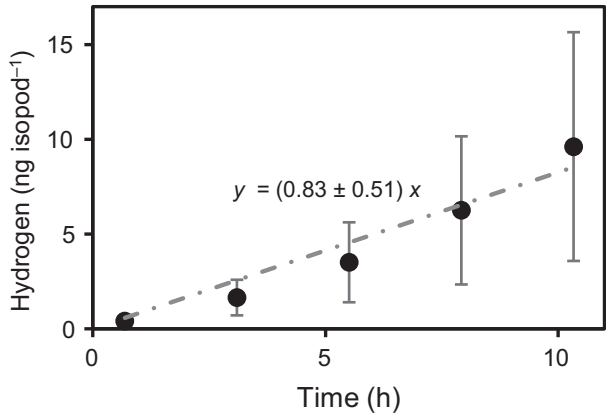

FIGURE 2 Hydrogen accumulation in the headspace of whole isopods incubated under air for 10 h. Values represent means of triplicate vials each containing three isopods that were analysed directly after collection and not subjected to a microplastic-exposure experiment. The dot-dashed line indicates a 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. The hydrogen emission rate of H_2 was 10.3 ± 6.3 nmol H_2 (g isopod fresh weight)$^{-1}$ h^{-1}.

Actinobacteria (mainly *Microbacteriaceae*), Bacteroida (mainly *Flavobacteriaceae*), Gammaproteobacteria (mainly *Enterobacteriaceae* and *Vibrionaceae*) and Verrucomicrobiae (mainly *Opitutaceae*) (Figure S8C). All of these taxa, but the Actinobacteria to a minor extent, were also found at 16S rRNA level (Figure S8D).

Gut communities differed from the food communities as indicated by principal coordinates analysis (PERMANOVA; $p < 0.005$; Figure S9A; for further details see Supplementary Results). ASVs assigned to *Vibrio rumoiensis* correlated well with gut communities. To elucidate the effects of MP, the data sets were analysed separately for the gut and the food communities (Figures 4 and S9B). In addition to *V. rumoiensis*, the gut communities were mainly affected by *Enterobacteriaceae* and *Microbacteriaceae* (Figure 4). PERMANOVA revealed that gut communities differed significantly due to MP-treatment. An effect of MP-treatment and dosage was obtained. For the latter, significant difference between communities exposed to 2.5% and 5% MP has been confirmed by a pairwise comparison (Table S3). Such pairwise comparisons essentially confirmed differences between gut communities of isopods fed with PLA-food and those fed with PET- or PS-food with low *p*-values ($p < 0.07$), but failed to confirm other MP-treatment effects ($p > 0.15$) (Table S4).

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

Most genera were shared among all treatments in isopod guts (43%–49%; Figures 5 and S10A). On 16S rRNA gene and 16S rRNA level, only a few genera were uniquely found in guts of isopods fed with PET- or PS-food (2%–4%), but more in case of guts of isopods fed with control- or PLA-food (7%–13%). Moreover, more genera in the guts of isopods exposed to control-food were shared with those exposed to PLA-food (8%–13%) than with those exposed to PET- (1%) or PS-food (0%). Genera within *Alcaligenaceae* were among exclusive taxa in guts of PLA-food exposed isopods.

Some taxa were also found to be significantly indicative in these guts (Figure 6; Tables S6 and S7). The majority of indicator genera were found in guts of isopods fed with PLA-food (8 out of 14 and 13 out of 21 genera on 16S rRNA gene and 16S rRNA level, respectively) and none were found in those fed with PS-food. The communities in guts of isopods fed with control-food were more similar to those fed with PLA-food than to those fed with PET- or PS-food, as the former shared more indicator taxa. In particular, most of

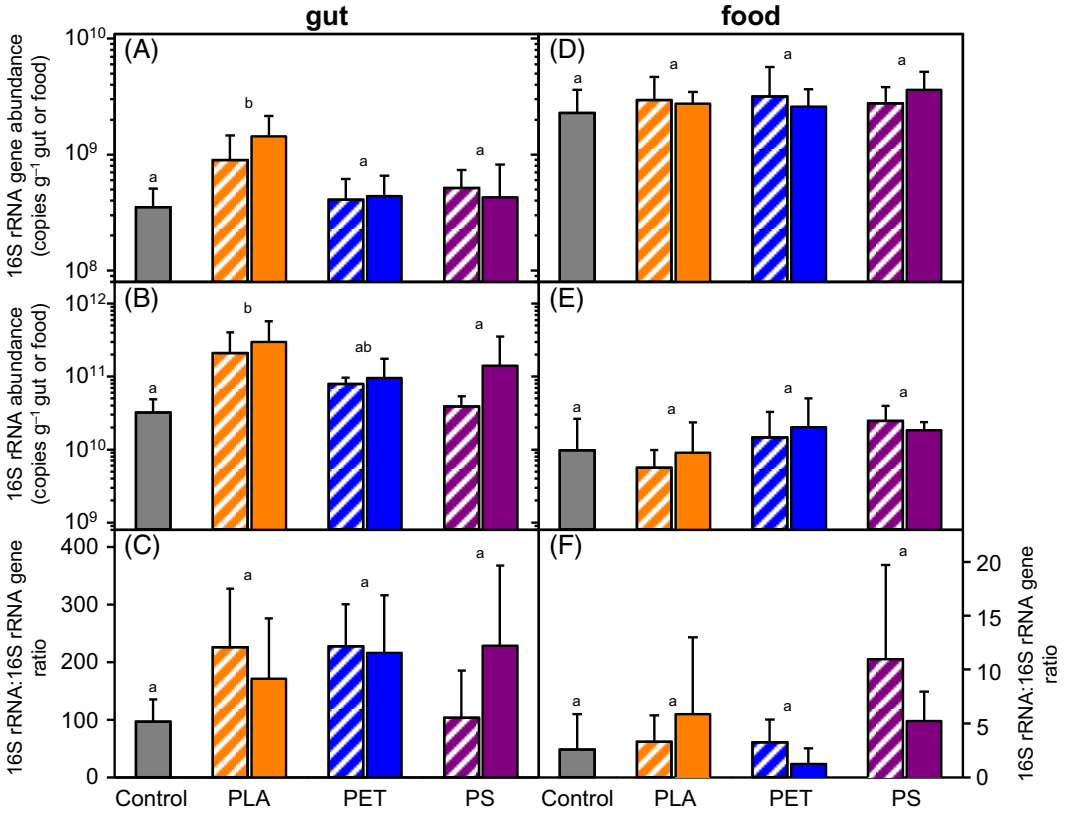

FIGURE 3 Effects of microplastic (MP) ingestion on the abundance of bacterial 16S rRNA genes and 16S rRNA in the gut and respective food of *Porcellio scaber*. Nucleic acid extracts derived from the guts of isopods (A–C) that were exposed to control- or 2.5%-MP- (striped) or 5%-MP- (no pattern) food pellets (D–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 were corrected for the proportion on endosymbionts obtained from sequencing analysis (see Figure 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. PET, polyethylene terephthalate; PLA, polylactic acid; PS, polystyrene.

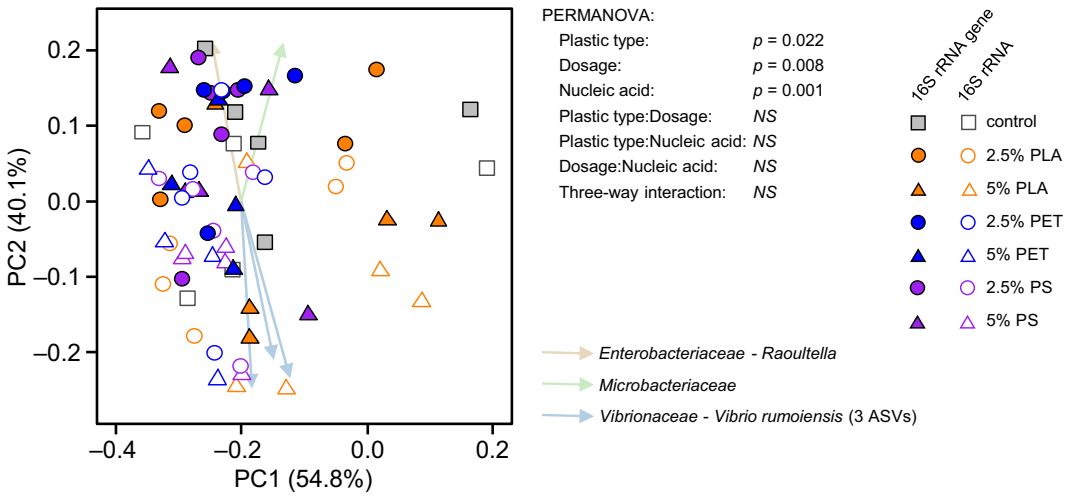

FIGURE 4 Beta diversity of the active bacterial gut communities. Principal coordinates analysis plots are based on Aitchison distance matrixes derived from analyses of the 16S rRNA genes and 16S rRNA. Results of the PERMANOVA are given for each plot. Arrows represent amplicon sequencing variants (ASVs) assigned on family and genus/species level (if applicable) that were highly correlated with the separation of samples. PET, polyethylene terephthalate; PLA, polylactic acid; PS, polystyrene.

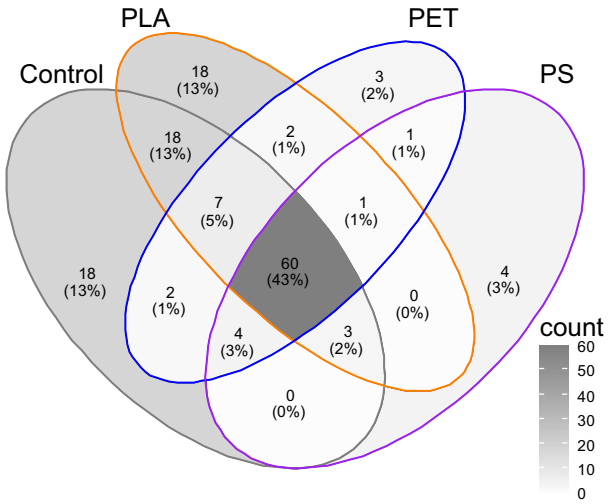

FIGURE 5 Shared and unique numbers and proportions of taxa among guts of isopods fed with control-, polylactic acid (PLA)-, polyethylene terephthalate (PET)- and polystyrene (PS)-food on 16S rRNA level. Only taxa on genus level (if applicable) that occur in at least 30% of the replicates were included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

these genera were more abundant in guts of isopods fed with PLA-food than in those fed with control-food. *Chryseobacterium*, *Devosia*, *Niabella, Prosthecobacter*, *Taeseokella* and uncultured Rhodospirillales were among the indicator taxa on 16S rRNA level in guts of isopods fed with PLA-food (Figure 6A; Table S7). In guts of isopods fed with PET-food, *Legionella*, *Microbacterium*, *Mycobacterium*, *Paenibacillus* and at least three different genera within the *Enterobacteriaceae* were attributed to indicator genera on 16S rRNA level (Figure 6B, Table S7).

In addition, MP affected food communities. More exclusive genera were obtained in the PS-food than in other kind of food-pellets (Figure S10B,C). Accordingly, more indicator taxa were found in the PS-food with most of them belonging to the Rhizobiales (Tables S8 and S9). For the control- or PLA-food, no indicators were found. Notably, none of the MP-specific indicator taxa found in the guts were reflected in the respective food-pellets (Tables S7–S10). Such a finding was supported by comparing the abundance of MP-specific indicator taxa from the guts with their abundance in the food pellets (Figures 6, S11 and S12).

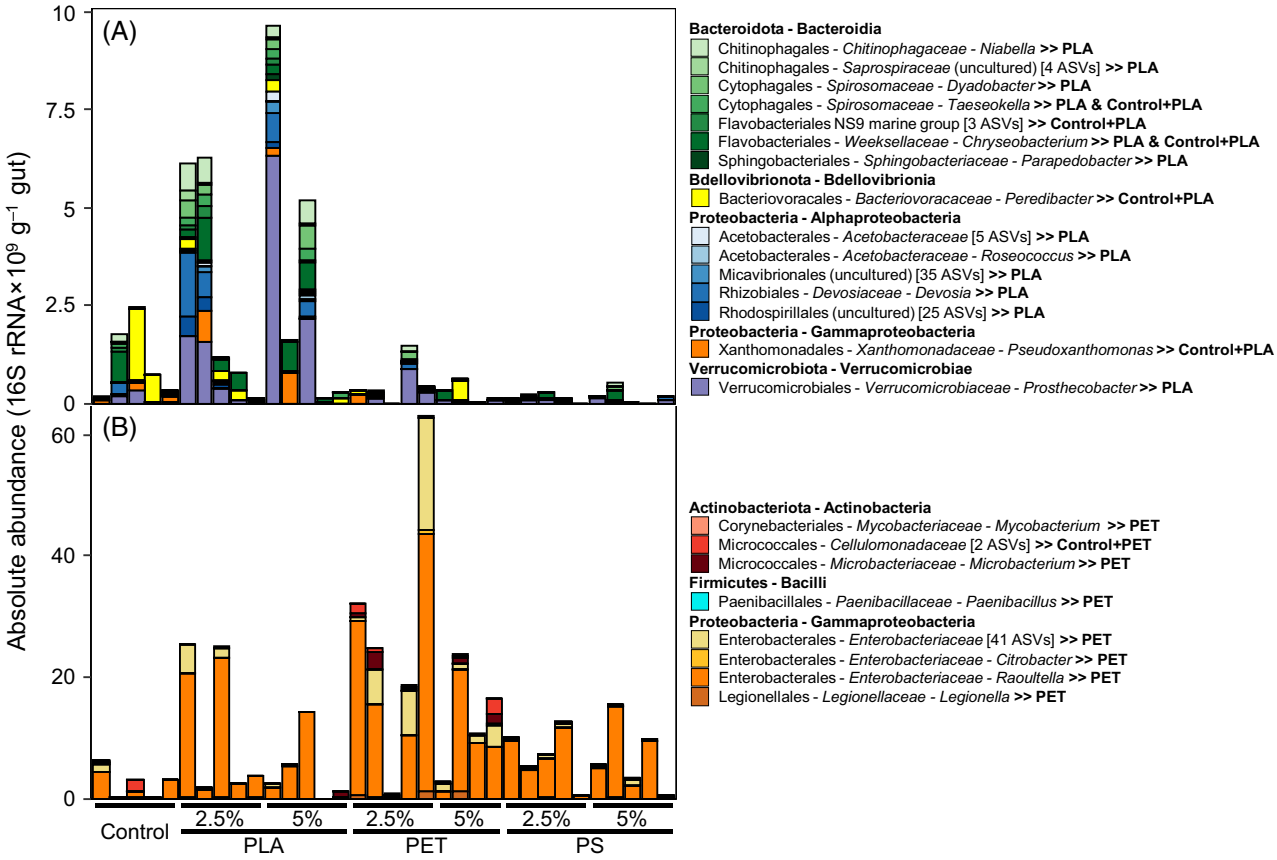

FIGURE 6 Indicator taxa in isopod guts on 16S rRNA level. The relative abundances of taxa were normalized with the total 16S rRNA abundance derived from qPCR analysis. Indicator taxa for the polylactic acid (PLA)- (and control-) (A) or polyethylene terephthalate (PET)- (and control-) (B) treatments were identified. Each bar represents one gut. Only taxa on genus level (if applicable, otherwise lowest classification and the number of amplicon sequencing variants [ASVs] are given) that occur in at least three replicates were accepted as potential indicators. As reference, numbers of absolute abundances are also given in Table S7.

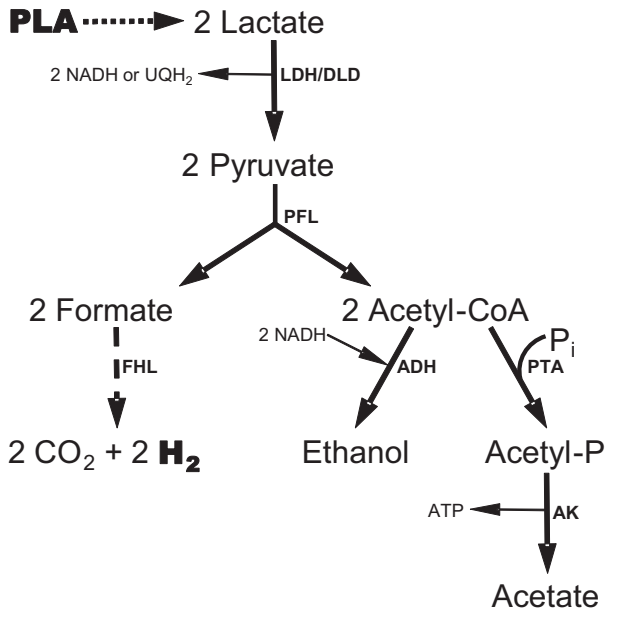

FIGURE 7 Potential mixed acid fermentation pathway by *Enterobacteriaceae* initiated with lactate. Under conditions, at which pyruvate generated from glucose (or similar compounds) is limited and lactate (that could be derived from polylactic acid [PLA] as indicated by a dotted arrow) is available, two lactate are oxidized by lactate dehydrogenase (LDH or DLD) yielding two pyruvate and two NADH or ubiquinol, respectively. Two pyruvate react to two acetyl-CoA and two formate catalysed by pyruvate formate lyase (PFL). One acetyl-CoA is then converted to acetate via phosphotransacetylase (PTA) and acetate kinase (AK) yielding one ATP, the other acetyl-CoA is reduced to ethanol by alcohol dehydrogenase (ADH) thereby using two NADH. Formate hydrogen lyase (FHL) transforms formate to carbon dioxide and molecular hydrogen preferentially under acidic conditions (indicated by a dashed arrow).

DISCUSSION

Isopods are globally abundant detritivores with a rarely studied gut environment and microbiota, as well as an unknown relevance for atmospheric trace gas emissions. Effects of MP pollution on model detritivores are largely unclear to date. Moreover, the effect of biodegradable MPs has not been investigated previously. Here we provide new insights into effects of biodegradable (PLA) and non-biodegradable (PS and PET) MP polymer types on the gut microbial community as well as activity of the model isopod *P. scaber*, and identify *P. scaber* as a MP-impacted mobile source of molecular hydrogen. We extend previous studies on the effect of PE, tire particles or polyester fibres on life history traits in *P. scaber* that revealed no or only marginal fitness effects (Jemec Kokalj et al., 2018; Selonen et al., 2020, 2021), which is in line with the findings of this study. *P. scaber* was not affected by the ingestion of MPs with its food as neither mortality nor weight gain or locomotor activity was altered (Figures S2 and S3). However, when given a choice between control- and MP-food, the isopods significantly avoided food containing PS. The palatability of the food source coheres with its microbial composition (Hassall et al., 1987; Ihnen & Zimmer, 2008) and therefore it can be considered that PS-food was less attractive, as indeed, most differences of the microbial composition were found between control- and PS-food (Figure S9). Avoidance behaviour of isopods is commonly observed against metals, pesticides, pharmaceuticals or chars, and already at low concentrations, it is often a more sensitive measure for adverse effects compared with fitness parameters (Loureiro et al., 2005; Madžarić et al., 2018; Tourinho et al., 2015; Zidar et al., 2019; Žižek & Zidar, 2013). Effects of long-term exposure to MP-contaminated food sources on the isopods' fitness have not been addressed and cannot be excluded.

Despite the importance of the gut microbiota in soil invertebrates, previous studies testing MP effects on invertebrates are limited (Brandon et al., 2018; Zhu, Chen, et al., 2018; Zhu, Fang, et al., 2018) and are absent in the case of *P. scaber*. In this study, analyses were performed on 16S rRNA gene and 16S rRNA level with the former reflecting the present community and the latter the rather active part of this community, which is commonly a more sensitive response measure. Findings regarding the general gut and food microbiota (MP-treatment independent) are discussed in the Supplementary Discussion. The diversity of bacterial gut communities of isopods exposed to MPs differed with respect to exclusive and indicative taxa (Figures 5 and 6; Tables S6–S9). These differences were not related to differences within the food communities (Figures S10–S12). Generally, the gut communities of the isopods fed with MP-free control-food shared more taxa and indicators with those fed with PLA-food than with PS- or PET-food. Moreover, PLA-food increased bacterial proliferation as growth (16S rRNA gene level) and activity (16S rRNA level) were stimulated (Figure 3). This suggests that some PLA has been degraded in the isopod guts. Abiotic degradation of PLA occurs due to hydrolysis of ester linkages releasing lactic acid at pH 4–7 at low rates (Belbella et al., 1996; deJong et al., 2001; Lyu & Untereker, 2009). The moderately acidic pH inside the gut would thus allow for some abiotic, acid-catalysed PLA hydrolysis. However, such abiotic hydrolysis takes several months or even years at environmentally relevant temperatures (<30°C; Lunt, 1998). Biotic PLA degradation is enhanced by enzymatic cleavage of ester bonds and depolymerization of the polymer to oligomers, dimers and lactic acid monomers (Sander, 2019). Various taxa possess extracellular hydrolytic, PLA depolymerizing enzymes like certain lipases, carboxylesterases and proteinases (Hajighasemi et al., 2016; Zaaba & Jaafar, 2020). Many of such enzymes are active at the gut pH of greater than or equal to 5 (Hajighasemi et al., 2016; Skowron et al., 2020), suggesting microbial, enzyme-catalysed

rather than abiotic PLA hydrolysis to lactate in the gut of isopods. Subsequent fermentation of lactate in the anoxic gut environment is likely (Seeliger et al., 2002; Ohnishi et al., 2012).

Indeed, some genera within Actinobacteria, which were ubiquitous in the isopod guts and less abundant in the food pellets (Figure S8; Supplementary Discussion) are possible PLA degraders, as numerous members of this class are capable of PLA degradation (Butbunchu & Pathom-Aree, 2019). Actinobacteria include aerobes as well as anaerobes and may subsist in the mainly anoxic isopod guts (Servin et al., 2008). Members of *Alcaligenaceae* were exclusively found in the guts of isopods fed with PLA-food suggesting at least a minor activation by PLA. *Alcaligenes* sp. of the *Alcaligenaceae* are well known to produce lipases that might contribute to PLA hydrolysis (Hoshino & Isono, 2002; Oda et al., 1997). The absence of a massive stimulation of such taxa in PLA treatments in spite of a potential PLA hydrolysis activity might be due to the lack of a specialized metabolism necessary for energy conservation from lactate under anoxic conditions. *Saprospiraceae* (*Parapedobacter*), *Micavibrionaceae* and *Saccharimonadaceae* (TM7) were indicators for guts of isopods exposed to PLA-food (Table S6). *Parapedobacter luteus* of the *Saprospiraceae* is capable of Tween 80 hydrolysis, which has structural similarities to PLA (Kim et al., 2010). *Micavibrionaceae* showed affinity for PLA-blended PBAT films (Meyer-Cifuentes et al., 2020), suggesting a possible role of both taxa in PLA hydrolysis. *Saccharimonadaceae* (also known as Saccharibacteria, TM7, or clade G6) show an anaerobic lifestyle, were enriched in soil with the structurally related polymer PBAT, are proposed to scavenge small molecular weight carbon during hydrocarbon degradation and host lactate dehydrogenases, suggesting their involvement in lactic acid removal during PLA degradation (Baker, 2021; Figueroa-Gonzalez et al., 2020; Li et al., 2022; Qiu et al., 2014). Additionally, *Xanthomonadaceae* were found in an anaerobic sludge incubation supplemented with PLA (Yagi et al., 2014), suggesting that *Pseudoxanthomonas*, an active genus indicative for guts of PLA-exposed isopods, may have contributed to PLA degradation (Figure 6). Further most abundant indicators here were *Chryseobacterium*, *Devosia*, *Niabella*, *Prosthecobacter*, *Taeseokella* and uncultured Rhodospirillales. Potential PLA degradation capabilities of these taxa are currently unknown but cannot be excluded and they may also possess enzymes capable of cleaving the ester bonds of PLA. However, they may have also taken advantage from enhanced lactic acid release during PLA degradation. A facultative lifestyle is conceivable for all of them (Hedlund et al., 1997; Imhoff et al., 2005; Kämpfer et al., 2011; McBride et al., 2014; Wu et al., 2013; Yoon et al., 2007).

A further novelty of this study was the assessment of in situ hydrogen production in the isopod guts via microsensor measurements. So far such hydrogen production has only been shown for a few other soil-dwelling invertebrates, such as earthworms (*Lumbricus terrestris*; Wüst et al., 2009), termites (*Cryptotermes secundus, Reticulitermes flavipes*, *Reticulitermes santonensis, Zootermopsis nevadensis*; Ebert & Brune, 1997; Pester & Brune, 2007), cockroaches (*Shelfordella lateralis*; Schauer et al., 2012) and millipedes (*Archispirostreptus gigas, Epibolus pulchripes*; Horváthová et al., 2021). Hydrogen production in the guts of isopods exposed to PLA-food was higher than in the other guts (Figures 1 and S6, Table S1), suggesting higher fermentative activities in the presence of PLA. Degradation of PLA generates lactate (see above), which can then be fermented to acetate, propionate, carbon dioxide and water, or hydrogen (Seeliger et al., 2002; Ohnishi et al., 2012). Obligate anaerobes like *Clostridiaceae* are capable of fermenting acetate and propionate to hydrogen and carbon dioxide, but were absent from isopod guts (Figure S8). Moreover, other hydrogen-producing fermenting gut bacteria, that are often obligate anaerobes (Schmidt et al., 2010), were not identified by the sequencing analyses (Figure S8) and [Fe-Fe]-hydrogenase genes (in the genomic repertoire of these organisms) were not PCR-amplifiable. The absence of obligate anaerobes in the gut system is somewhat surprising compared with other anoxic gut systems (Arumugam et al., 2011; Ebert & Brune, 1997; Wüst et al., 2009; Yun et al., 2014) and probably owed to the short gut passage time of ≈5 h not allowing for the proliferation of such organisms from ingested inactive forms (Clegg et al., 1994). However, the short gut passage time will suffice for the activation of fermentation by facultatives. *Enterobacteriacea* are facultatives, well known to produce hydrogen via mixed acid fermentation. An explanation for the stimulated hydrogen production in PLA-fed isopod guts is thus a variation of the mixed acid fermentation pathway by *Enterobacteriaceae* generating ethanol, acetate and formate, and associated formate hydrogen lyase (FHL) catalysed hydrogen production (Figure 7; Clark, 1989). A 'proof of principle' experiment using *Escherichia coli* as a model organism of the *Enterobacteriaceae* has indeed revealed that hydrogen was generated from lactate (see Supplementary Experimental Procedures and Results; Figure S13). A pH of 5–6 in the medial part of the gut, where hydrogen production was high (Figures 1, S5 and S6), represents favourable conditions for FHL gene expression (which is induced at low pH and the presence of formate) and thus formate transformation (Clark, 1989). *Enterobacteriacea* were ubiquitous as well as active in *P. scaber* guts as was hydrogen production. Energy conservation via the variation of the mixed acid fermentation pathway was likely negligible, leading to undetectable stimulation in growth

or activity and may explain why *Enterobacteriacea* were not identified as indicators for the PLA-fed isopod guts.

In contrast, little hydrogen was produced in the guts of isopods fed with PET- and PS-food. Hydrogen consumption by obligate anaerobic methanogens was unlikely to be the reason, as amplification targeting archaeal 16S rRNA failed and methane was not produced (data not shown), when whole isopods were analysed. However, two reasons are conceivable: Either the MPs had inhibitory effects on fermentative microorganisms, or Knallgas bacteria were stimulated and consumed most of the hydrogen. Some evidence is given for the latter: *Mycobacterium*, an indicator for the gut of isopods fed with PET-food (Table S8), has been identified as a hydrogen-oxidizing bacterium with oxygen as electron acceptor (Osborne et al., 2010). Oxygen diffusing in the isopod guts from the gut wall might enable hydrogen consumption leading to immediate consumption, which is well known in termites (Brune et al., 1995). Nevertheless, the actual reason of reduced hydrogen emission remains to be determined.

Hydrogen is a valuable electron donor fuelling hydrogen-oxidizing processes either inside or outside the gut and MP contamination may have consequences for microbial food webs and global hydrogen emissions. *P. scaber* hydrogen concentrations in the centre of the gut ranged from 5 to 30 μM and were thus in the range of those from *L. terrestris* and *R. flavipes*, demonstrating *P. scaber*'s high hydrogen emission potential (Ebert & Brune, 1997; Wüst et al., 2009). Hydrogen emissions from whole isopods were variable, on average 0.83 ± 0.51 ng isopod^{-1} h^{-1} (equivalent to 10.3 ± 6.3 nmol H_2 (g isopod fresh weight)$^{-1}$ h^{-1}; Figure 2), which is likewise in the same range as hydrogen emissions from earthworms (6.3 ± 4.8 nmol H_2 (g worm fresh weight)$^{-1}$ h^{-1}; Wüst et al., 2009) and termites (<0.5–80 nmol H_2 (g termite fresh weight)$^{-1}$ h^{-1}; Pester & Brune, 2007). Assuming that 20% of the Earth's terrestrial ecosystems (total surface area of 1.5×10^{14} m^2; Schlesinger, 1997) are colonized by these cosmopolitan isopods with a density of 75 isopods m^{-2} (median of distributions given in Paoletti & Hassall, 1999), the annual contribution of *P. scaber* to the global hydrogen production is approximately 0.6–2.6×10^7 kg year^{-1}. This value is in the same range of what is annually emitted from paddy fields (1.3×10^7 kg year^{-1}; Koyama, 1963). Given the experimental conditions under which the hydrogen emission potentials were obtained, the contribution of *P. scaber* to the global hydrogen production is rather underestimated. It is common knowledge that oxygen inhibits hydrogen production by anaerobic fermentation processes. The actual oxygen partial pressure during the experiment, at which the hydrogen emission rates from whole isopods were determined, were higher than naturally (0.35 bar rather than 0.21 bar, because the 3-mL vials were pressurized by injection of 2 mL air). Thus, we provide a conservative estimate of the global importance of isopods for terrestrial hydrogen emissions.

Taken together, this study provides new insights regarding the effects of MP on soil invertebrates that are potentially affected by MP-ingestion in the longer term and highlights the hitherto unknown hydrogen emitting capacity of a widely distributed group of detritivores. We identified the moderately acidic, anoxic, medial and posterior parts of the isopod's gut as 'hot spots' for hydrogen production. Such a hydrogen production was stimulated by PLA- and inhibited by PET- and PS-ingestion, which was concomitant to changes in the composition of the gut microbiota and in agreement with our initial hypothesis that biodegradable and non-biodegradable MPs have contrasting effects. The nature of low hydrogen emissions in response to PET and PS exposure remains speculative, as are consequences of altered hydrogen metabolism inside and outside the isopod gut, opening up new avenues for future research.

AUTHOR CONTRIBUTIONS

Linda Hink: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (equal); methodology (lead); software (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Anja Holzinger:** Conceptualization (equal); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); validation (equal); writing – review and editing (equal). **Tobias Sandfeld:** Conceptualization (supporting); formal analysis (supporting); investigation (supporting); methodology (equal); writing – review and editing (supporting). **Alfons R. Weig:** Data curation (equal); formal analysis (equal); investigation (supporting); software (equal); writing – review and editing (equal). **Andreas Schramm:** Supervision (equal); writing – review and editing (equal). **Heike Feldhaar:** Conceptualization (equal); funding acquisition (lead); project administration (equal); supervision (equal); writing – review and editing (equal). **Marcus A. Horn:** Conceptualization (lead); funding acquisition (lead); investigation (equal); project administration (lead); supervision (lead); writing – original draft (equal); writing – review and editing (lead).

ACKNOWLEDGEMENTS

This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation); Project Number 391977956; SFB 1357 Microplastic subproject A02. We thank Peter Strohriegl and Lisa Weber for processing polymer granules, Lars Borregard Pedersen for microsensor construction and assistance during measurements, and the Poul Due Jensen Foundation for funding the sensor work. Alina Bernstein

and Sabrina Kaupp helped to perform the isopod feeding experiments. We are also grateful to Anja Poehlein for library preparation and sequencing. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Amplicon sequencing data have been deposited in the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) under the Bioproject PRJNA832915.

ORCID


Marcus A. Horn https://orcid.org/0000-0001-8510-9651

REFERENCES

Andrady, A.L. (2017) The plastic in microplastics: a review. *Marine Pollution Bulletin*, 119, 12–22.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R. et al. (2011) Enterotypes of the human gut microbiome. *Nature*, 473, 174–180.

Baker, J.L. (2021) Complete genomes of clade G6 *Saccharibacteria* suggest a divergent ecological niche and lifestyle. *mSphere*, 6, e0053021.

Belbella, A., Vauthier, C., Fessi, H., Devissaguet, J.-P. & Puisieux, F. (1996) In vitro degradation of nanospheres from poly(D,L-lactides) of different molecular weights and polydispersities. *International Journal of Pharmaceutics*, 129, 95–102.

Bouchon, D., Zimmer, M. & Dittmer, J. (2016) The terrestrial isopod microbiome: An all-in-one toolbox for animal–microbe interactions of ecological relevance. *Frontiers in Microbiology*, 7, 1472.

Brandon, A.M., Gao, S.-H., Tian, R., Ning, D., Yang, S.-S., Zhou, J. et al. (2018) Biodegradation of polyethylene and plastic mixtures in mealworms (larvae of *Tenebrio molitor*) and effects on the gut microbiome. *Environmental Science & Technology*, 52, 6526–6533.

Brune, A., Emerson, D. & Breznak, J.A. (1995) The termite gut microflora as an oxygen sink: microelectrode determination of oxygen and pH gradients in guts of lower and higher termites. *Applied and Environmental Microbiology*, 61, 2681–2687.

Büks, F. & Kaupenjohann, M. (2022) What comes after the Sun? On the integration of soil biogeochemical pre-weathering into microplastic experiments. *The Soil*, 8, 373–380.

Büks, F., van Loes Schaik, N. & Kaupenjohann, M. (2020) What do we know about how the terrestrial multicellular soil fauna reacts to microplastic? *The Soil*, 6, 245–267.

Butbunchu, N. & Pathom-Aree, W. (2019) Actinobacteria as promising candidate for polylactic acid type bioplastic degradation. *Frontiers in Microbiology*, 10, 2834.

Clark, D.P. (1989) The fermentation pathways of *Escherichia coli*. *FEMS Microbiology Letters*, 63, 223–234.

Clegg, C.D., van Elsas, J.D., Anderson, J.M. & Lappin-Scott, H.M. (1994) Assessment of the role of a terrestrial isopod in the survival of a genetically modified pseudomonad and its detection using the polymerase chain reaction. *FEMS Microbiology Ecology*, 15, 161–168.

de Cáceres, M. & Legendre, P. (2009) Associations between species and groups of sites: indices and statistical inference. *Ecology*, 90, 3566–3574.

de Jong, S.J., Arias, E.R., Rijkers, D., van Nostrum, C.F., Kettenes-van den Bosch, J.J. & Hennink, W.E. (2001) New insights into the hydrolytic degradation of poly(lactic acid): participation of the alcohol terminus. *Polymer*, 42, 2795–2802.

Dolar, A., Drobne, D., Dolenec, M., Marinšek, M. & Jemec Kokalj, A. (2022) Time-dependent immune response in *Porcellio scaber* following exposure to microplastics and natural particles. *Science of the Total Environment*, 818, 151816.

Dolar, A., Selonen, S., van Gestel, C.A.M., Perc, V., Drobne, D. & Jemec Kokalj, A. (2021) Microplastics, chlorpyrifos and their mixtures modulate immune processes in the terrestrial crustacean *Porcellio scaber*. *Science of the Total Environment*, 772, 1–11.

Drobne, D. (1995) Bacteria adherent to the hindgut of terrestrial isopods. *Acta Microbiologica et Immunologica Hungarica*, 42, 45–52.

Ebert, A. & Brune, A. (1997) Hydrogen concentration profiles at the oxic-anoxic interface: a microsensor study of the hindgut of the wood-feeding lower termite *Reticulitermes flavipes* (Kollar). *Applied and Environmental Microbiology*, 63, 4039–4046.

Edney, E.B. (1954) Woodlice and the land habitat. *Biological Reviews of the Cambridge Philosophical Society*, 29, 185–219.

Emadian, S.M., Onay, T.T. & Demirel, B. (2017) Biodegradation of bioplastics in natural environments. *Waste Management*, 59, 526–536.

Figueroa-Gonzalez, P.A., Bornemann, T.L.V., Adam, P.S., Plewka, J., Révész, F., von Hagen, C.A. et al. (2020) Saccharibacteria as organic carbon sinks in hydrocarbon-fueled communities. *Frontiers in Microbiology*, 11, 587782.

Gambarini, V., Pantos, O., Kingsbury, J.M., Weaver, L., Handley, K. M. & Lear, G. (2021) Phylogenetic distribution of plastic-degrading microorganisms. *mSystems*, 6, 1–13.

Gao, C.H., Yu, G. & Cai, P. (2021) ggVennDiagram: an intuitive, easy-to-use, and highly customizable R package to generate Venn Diagram. *Frontiers in Genetics*, 12, 706907.

Geyer, R., Jambeck, J.R. & Law, K.L. (2017) Production, use, and fate of all plastics ever made. *Science Advances*, 3, e1700782.

Gkoutselis, G., Rohrbach, S., Harjes, J., Obst, M., Brachmann, A., Horn, M.A. et al. (2021) Microplastics accumulate fungal pathogens in terrestrial ecosystems. *Scientific Reports*, 11, 13214.

Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G. & Bailey, M.J. (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. *Applied and Environmental Microbiology*, 66, 5488–5491.

Hajighasemi, M., Nocek, B.P., Tchigvintsev, A., Brown, G., Flick, R., Xu, X. et al. (2016) Biochemical and structural insights into enzymatic depolymerization of polylactic acid and other polyesters by microbial carboxylesterases. *Biomacromolecules*, 17, 2027–2039.

Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Daugaard, A.E. et al. (2019) Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. *Environmental Science & Technology*, 53, 1039–1047.

Hassall, M., Turner, J.G. & Rands, M.R.W. (1987) Effects of terrestrial isopods on the decomposition of woodland leaf litter. *Oecologia*, 72, 597–604.

Hedlund, B.P., Gosink, J.J. & Staley, J.T. (1997) Verrucomicrobia div. nov., a new division of the bacteria containing three new species of *Prosthecobacter*. *Antonie Van Leeuwenhoek*, 72, 29–38.

Herlemann, D.P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J. & Andersson, A.F. (2011) Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *The ISME Journal*, 5, 1571–1579.

Ho, A., Mendes, L.W., Lee, H.J., Kaupper, T., Mo, Y., Poehlein, A. et al. (2020) Response of a methane-driven interaction network to stressor intensification. *FEMS Microbiology Ecology*, 96, fiaa180.

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E. & Svendsen, C. (2017) Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. *Science of the Total Environment*, 586, 127–141.

Horváthová, T., Babik, W. & Bauchinger, U. (2016) Biofilm feeding: microbial colonization of food promotes the growth of a detritivorous arthropod. *Zookeys*, 577, 25–41.

Horváthová, T., Babik, W., Kozłowski, J. & Bauchinger, U. (2019) Vanishing benefits – the loss of actinobacterial symbionts at elevated temperatures. *Journal of Thermal Biology*, 82, 222–228.

Horváthová, T., Šustr, V., Chroňáková, A., Semanová, S., Lang, K., Dietrich, C. et al. (2021) Methanogenesis in the digestive tracts of the tropical millipedes *Archispirostreptus gigas* (Diplopoda, Spirostreptidae) and *Epibolus pulchripes* (Diplopoda, Pachybolidae). *Applied and Environmental Microbiology*, 87, e0061421.

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

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, 50, 346–363.

Huerta Lwanga, E., Mendoza Vega, J., Ku Quej, V., Chi, J.d.L.A., Del Sanchez Cid, L., Chi, C. et al. (2017) Field evidence for transfer of plastic debris along a terrestrial food chain. *Scientific Reports*, 7, 14071.

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

Imhoff, J.F., Hiraishi, A. & Sülling, J. (2005) Anoxygenic phototrophic purple bacteria. In: Brenner, D.J., Krieg, N.R., Staley, J.R. & Garrity, G.M. (Eds.) *Bergey's manual of systematic bacteriology. Volume two: the Proteobacteria. Part A. Introductory essays*. New York: Springer, pp. 119–132.

Jemec Kokalj, A., Horvat, P., Skalar, T. & Kržan, A. (2018) Plastic bag and facial cleanser derived microplastic do not affect feeding behaviour and energy reserves of terrestrial isopods. *Science of the Total Environment*, 615, 761–766.

Ji, Z., Huang, Y., Feng, Y., Johansen, A., Xue, J., Tremblay, L.A. et al. (2021) Effects of pristine microplastics and nanoplastics on soil invertebrates: a systematic review and meta-analysis of available data. *Science of the Total Environment*, 788, 147784.

Ju, H., Zhu, D. & Qiao, M. (2019) Effects of polyethylene microplastics on the gut microbial community, reproduction and avoidance behaviors of the soil springtail, *Folsomia candida*. *Environmental Pollution*, 247, 890–897.

Kämpfer, P., Lodders, N. & Falsen, E. (2011) *Hydrotalea flava* gen. nov., sp. nov., a new member of the phylum Bacteroidetes and allocation of the genera *Chitinophaga*, *Sediminibacterium*, *Lacibacter*, *Flavihumibacter*, *Flavisolibacter*, *Niabella*, *Niastella*, *Segetibacter*, *Parasegetibacter*, *Terrimonas*, *Ferruginibacter*, *Filimonas* and *Hydrotalea* to the family *Chitinophagaceae* fam. Nov. *International Journal of Systematic and Evolutionary Microbiology*, 61, 518–523.

Kim, S.-J., Weon, H.-Y., Kim, Y.-S., Yoo, S.-H., Kim, B.-Y., Anandham, R. et al. (2010) *Parapedobacter luteus* sp. nov. and *Parapedobacter composti* sp. nov., isolated from cotton waste compost. *International Journal of Systematic and Evolutionary Microbiology*, 60, 1849–1853.

Kirstein, I.V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Löder, M. et al. (2016) Dangerous hitchhikers? Evidence for potentially pathogenic *Vibrio* spp. on microplastic particles. *Marine Environmental Research*, 120, 1–8.

Kostanjsek, R., Lapanje, A., Rupnik, M., Strus, J., Drobne, D. & Avgustin, G. (2004) Anaerobic bacteria in the gut of terrestrial isopod crustacean *Porcellio scaber*. *Folia Microbiologica*, 49, 179–182.

Kostanjsek, R., Strus, J. & Avgustin, G. (2002) Genetic diversity of bacteria associated with the hindgut of the terrestrial crustacean *Porcellio scaber* (Crustacea: isopoda). *FEMS Microbiology Ecology*, 40, 171–179.

Koyama, T. (1963) Gaseous metabolism in lake sediments and paddy soils and the production of atmospheric methane and hydrogen. *Journal of Geophysical Research*, 68, 3971–3973.

Lebreton, L., Egger, M. & Slat, B. (2019) A global mass budget for positively buoyant macroplastic debris in the ocean. *Scientific Reports*, 9, 12922.

Li, C., Cui, Q., Li, Y., Zhang, K., Lu, X. & Zhang, Y. (2022) Effect of LDPE and biodegradable PBAT primary microplastics on bacterial community after four months of soil incubation. *Journal of Hazardous Materials*, 429, 128353.

Loureiro, S., Soares, A.M.V.M. & Nogueira, A.J.A. (2005) Terrestrial avoidance behaviour tests as screening tool to assess soil contamination. *Environmental Pollution*, 138, 121–131.

Lunt, J. (1998) Large-scale production, properties and commercial applications of polylactic acid polymers. *Polymer Degradation and Stability*, 59, 145–152.

Lyu, S. & Untereker, D. (2009) Degradability of polymers for implantable biomedical devices. *International Journal of Molecular Sciences*, 10, 4033–4065.

Madžarić, S., Kos, M., Drobne, D., Hočevar, M. & Jemec Kokalj, A. (2018) Integration of behavioral tests and biochemical biomarkers of terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea) is a promising methodology for testing environmental safety of chars. *Environmental Pollution*, 234, 804–811.

Mangiafico, S. & Mangiafico, M.S. (2017) Package 'rcompanion'. *Cran Repository*, 20, 1–71.

Martino, C., Morton, J.T., Marotz, C.A., Thompson, L.R., Tripathi, A., Knight, R. et al. (2019) A novel sparse compositional technique reveals microbial perturbations. *mSystems*, 4, 13.

McBride, M.J., Liu, W., Lu, X., Zhu, Y. & Zhang, W. (2014) The family *Cytophagaceae*. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E. & Thompson, F. (Eds.) *The prokaryotes*. Berlin: Springer, pp. 577–593.

McMurdie, P.J. & Holmes, S. (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, 8, e61217.

Meyer-Cifuentes, I.E., Werner, J., Jehmlich, N., Will, S.E., Neumann-Schaal, M. & Öztürk, B. (2020) Synergistic biodegradation of aromatic-aliphatic copolyester plastic by a marine microbial consortium. *Nature Communications*, 11, 5790.

Oda, Y., Oida, N., Urakami, T. & Tonomura, K. (1997) Polycaprolactone depolymerase produced by the bacterium *Alcaligenes faecalis*. *FEMS Microbiology Letters*, 152, 339–343.

Ohnishi, A., Hasegawa, Y., Abe, S., Bando, Y., Fujimoto, N. & Suzuki, M. (2012) Hydrogen fermentation using lactate as the sole carbon source: solution for 'blind spots' in biofuel production. *RSC Advances*, 2, 8332.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D. et al. (2018) vegan: Community Ecology Package. Ordination methods, diversity analysis and other functions for community and vegetation ecologists, R package ver 2.5-7.

Osborne, C.A., Peoples, M.B. & Janssen, P.H. (2010) Detection of a reproducible, single-member shift in soil bacterial communities exposed to low levels of hydrogen. *Applied and Environmental Microbiology*, 76, 1471–1479.

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

Pester, M. & Brune, A. (2007) Hydrogen is the central free intermediate during lignocellulose degradation by termite gut symbionts. *The ISME Journal*, 1, 551–565.

Qiu, Y.-L., Kuang, X.-Z., Shi, X.-S., Yuan, X.-Z. & Guo, R.-B. (2014) *Terrimicrobium sacchariphilum* gen. nov., sp. nov., an anaerobic bacterium of the class 'Spartobacteria' in the phylum

Verrucomicrobia, isolated from a rice paddy field. *International Journal of Systematic and Evolutionary Microbiology*, 64, 1718–1723.

R Core Team. (2020) *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rabatin, S.C. & Stinner, B.R. (1988) Indirect effects of interactions between VAM fungi and soil-inhabiting invertebrates on plant processes. *Agriculture, Ecosystems and Environment*, 24, 135–146.

Revsbech, N.P. (1989) An oxygen microsensor with a guard cathode. *Limnology and Oceanography*, 34, 474–478.

Revsbech, N.P. & Jørgensen, B.B. (1986) Microelectrodes: their use in microbial ecology. In: Marshall, K.C. (Ed.) *Advances in microbial ecology*, Vol. 9. Boston, MA: Springer, pp. 293–352.

Rohrbach, S., Gkoutselis, G., Hink, L., Weig, A.R., Obst, M., Diekmann, A. et al. (2022) Microplastic polymer properties as deterministic factors driving terrestrial plastisphere microbiome assembly and succession in the field. *Environmental Microbiology*, 1–17. https://doi.org/10.1111/1462-2920.16234. Epub ahead of print.

Salazar, G. (2015) EcolUtils: utilities for community ecology analysis, R package ver 0.1.

Sander, M. (2019) Biodegradation of polymeric mulch films in agricultural soils: concepts, knowledge gaps, and future research directions. *Environmental Science & Technology*, 53, 2304–2315.

Schauer, C., Thompson, C.L. & Brune, A. (2012) The bacterial community in the gut of the cockroach *Shelfordella lateralis* reflects the close evolutionary relatedness of cockroaches and termites. *Applied and Environmental Microbiology*, 78, 2758–2767.

Schlesinger, W.H. (1997) *Biogeochemistry*, 2nd edition. San Diego, CA: Academic Press.

Schmidt, O., Drake, H.L. & Horn, M.A. (2010) Hitherto unknown Fe-Fe-hydrogenase gene diversity in anaerobes and anoxic enrichments from a moderately acidic fen. *Applied and Environmental Microbiology*, 76, 2027–2031.

Schramm, A. (2006) Microsensors for the study of microenvironments and processes in the intestine of invertebrates. In: König, H. & Varma, A. (Eds.) *Intestinal microorganisms of termites and other invertebrates*. Berlin, Heidelberg: Springer, pp. 463–473.

Seeliger, S., Janssen, P.H. & Schink, B. (2002) Energetics and kinetics of lactate fermentation to acetate and propionate via methylmalonyl-CoA or acrylyl-CoA. *FEMS Microbiology Letters*, 211, 65–70.

Selonen, S., Dolar, A., Jemec Kokalj, A., Sackey, L.N.A., Skalar, T., Cruz Fernandes, V. et al. (2021) Exploring the impacts of microplastics and associated chemicals in the terrestrial environment – exposure of soil invertebrates to tire particles. *Environmental Research*, 201, 111495.

Selonen, S., Dolar, A., Jemec Kokalj, A., Skalar, T., Parramon Dolcet, L., Hurley, R. et al. (2020) Exploring the impacts of plastics in soil – the effects of polyester textile fibers on soil invertebrates. *Science of the Total Environment*, 700, 134451.

Servin, J.A., Herbold, C.W., Skophammer, R.G. & Lake, J.A. (2008) Evidence excluding the root of the tree of life from the actinobacteria. *Molecular Biology and Evolution*, 25, 1–4.

Skowron, P.M., Krefft, D., Brodzik, R., Kasperkiewicz, P., Drag, M. & Koller, K.-P. (2020) An alternative for proteinase K-heat-sensitive protease from fungus *Onygena corvina* for biotechnology: cloning, engineering, expression, characterization and special application for protein sequencing. *Microbial Cell Factories*, 19, 135.

Therneau, T. (2015) A package for survival analysis in R, R package ver 2.7.

Tourinho, P.S., van Gestel, C.A.M., Jurkschat, K., Soares, A.M.V. M. & Loureiro, S. (2015) Effects of soil and dietary exposures to Ag nanoparticles and $AgNO_3$ in the terrestrial isopod *Porcellionides pruinosus*. *Environmental Pollution*, 205, 170–177.

van Gestel, C.A.M., Loureiro, S. & Idar, P. (2018) Terrestrial isopods as model organisms in soil ecotoxicology: a review. *Zookeys*, 801, 127–162.

Villanueva, R.A.M. & Chen, Z.J. (2019) ggplot2: elegant graphics for data analysis (2nd ed.). *Measurement: Interdisciplinary Research and Perspectives*, 17, 160–167.

Wang, Y. & Qian, P.-Y. (2009) Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS One*, 4, e7401.

Weig, A.R., Löder, M.G.J., Ramsperger, A.F.R. & Laforsch, C. (2021) *In situ* prokaryotic and eukaryotic communities on microplastic particles in a small headwater stream in Germany. *Frontiers in Microbiology*, 12, 660024.

Wu, Y.-F., Wu, Q.-L. & Liu, S.-J. (2013) *Chryseobacterium taihuense* sp. nov., isolated from a eutrophic lake, and emended descriptions of the genus *Chryseobacterium*, *Chryseobacterium taiwanense*, *Chryseobacterium jejuense* and *Chryseobacterium indoltheticum*. *International Journal of Systematic and Evolutionary Microbiology*, 63, 913–919.

Wüst, P.K., Horn, M.A. & Drake, H.L. (2009) *In situ* hydrogen and nitrous oxide as indicators of concomitant fermentation and denitrification in the alimentary canal of the earthworm *Lumbricus terrestris*. *Applied and Environmental Microbiology*, 75, 1852–1859.

Xing, D., Ren, N. & Rittmann, B.E. (2008) Genetic diversity of hydrogen-producing bacteria in an acidophilic ethanol-H_2-coproducing system, analyzed using the Fe-hydrogenase gene. *Applied and Environmental Microbiology*, 74, 1232–1239.

Yagi, H., Ninomiya, F., Funabashi, M. & Kunioka, M. (2014) Mesophilic anaerobic biodegradation test and analysis of eubacteria and archaea involved in anaerobic biodegradation of four specified biodegradable polyesters. *Polymer Degradation and Stability*, 110, 278–283.

Yoon, J.-H., Kang, S.-J., Park, S. & Oh, T.-K. (2007) *Devosia insulae* sp. nov., isolated from soil, and emended description of the genus *Devosia*. *International Journal of Systematic and Evolutionary Microbiology*, 57, 1310–1314.

Yoshida, N., Yagi, K., Sato, D., Watanabe, N., Kuroishi, T., Nishimoto, K. et al. (2005) Bacterial communities in petroleum oil in stockpiles. *Journal of Bioscience and Bioengineering*, 99, 143–149.

Yun, J.-H., Roh, S.W., Whon, T.W., Jung, M.-J., Kim, M.-S., Park, D.-S. et al. (2014) Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Applied and Environmental Microbiology*, 80, 5254–5264.

Zaaba, N.F. & Jaafar, M. (2020) A review on degradation mechanisms of polylactic acid: hydrolytic, photodegradative, microbial, and enzymatic degradation. *Polymer Engineering and Science*, 60, 2061–2075.

Zaman, M., Kleineidam, K., Bakken, L., Berendt, J., Bracken, C., Butterbach-Bahl, K. et al. (2021) Automated laboratory and field techniques to determine greenhouse gas emissions. In: Zaman, M., Heng, L. & Müller, C. (Eds.) *Measuring emission of agricultural greenhouse gases and developing mitigation options using nuclear and related techniques*. Cham: Springer International Publishing, pp. 109–139.

Zhu, B.-K., Fang, Y.-M., Zhu, D., Christie, P., Ke, X. & Zhu, Y.-G. (2018) Exposure to nanoplastics disturbs the gut microbiome in the soil oligochaete *Enchytraeus crypticus*. *Environmental Pollution*, 239, 408–415.

Zhu, D., Chen, Q.-L., An, X.-L., Yang, X.-R., Christie, P., Ke, X. et al. (2018) Exposure of soil collembolans to microplastics perturbs their gut microbiota and alters their isotopic composition. *Soil Biology and Biochemistry*, 116, 302–310.

Zidar, P., Kos, M., Ilič, E., Marolt, G., Drobne, D. & Jemec Kokalj, A. (2019) Avoidance behaviour of isopods (*Porcellio scaber*) exposed to food or soil contaminated with Ag^- and CeO_2^- nanoparticles. *Applied Soil Ecology*, 141, 69–78.

Zimmer, M. (2002) Nutrition in terrestrial isopods (Isopoda: Oniscidea): an evolutionary-ecological approach. *Biological Reviews of the Cambridge Philosophical Society*, 77, 455–493.

Zimmer, M. & Brune, A. (2005) Physiological properties of the gut lumen of terrestrial isopods (Isopoda: Oniscidea): adaptive to digesting lignocellulose? *Journal of Comparative Physiology. B*, 175, 275–283.

Zimmer, M. & Topp, W. (1998) Microorganisms and cellulose digestion in the gut of the woodlouse *Porcellio scaber*. *Journal of Chemical Ecology*, 24, 1397–1408.

Zižek, S., Hrženjak, R., Kalcher, G.T., Srimpf, K., Semrov, N. & Zidar, P. (2011) Does monensin in chicken manure from poultry farms pose a threat to soil invertebrates? *Chemosphere*, 83, 517–523.

Žižek, S. & Zidar, P. (2013) Toxicity of the ionophore antibiotic lasalocid to soil-dwelling invertebrates: avoidance tests in comparison to classic sublethal tests. *Chemosphere*, 92, 570–575.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hink, L., Holzinger, A., Sandfeld, T., Weig, A.R., Schramm, A., Feldhaar, H. et al. (2023) Microplastic ingestion affects hydrogen production and microbiomes in the gut of the terrestrial isopod *Porcellio scaber*. *Environmental Microbiology*, 1–16. Available from: https://doi.org/10.1111/1462-2920.16386