Accumulation of di-2-ethylhexyl phthalate from polyvinyl chloride flooring into settled house dust and the effect on the bacterial community

Samantha Velazquez [1] , **Chenyang Bi** [2,3] , **Jeff Kline** [1,4] , **Susie Nunez** [1] , **Rich Corsi** [3,5] , **Ying Xu** [3,6] , **Suzanne L Ishaq** [Corresp. 1,7]

[1] Biology and the Built Environment Center, University of Oregon, Eugene, Oregon, United States

[2] Department of Civil Engineering, Virginia Polytechnic Institute and State University (Virginia Tech), Blacksburg, Virginia, United States

[3] Department of Civil, Architectural and Environmental Engineering, University of Texas at Austin, Austin, Texas, United States

[4] Energy Studies and Buildings Laboratory, University of Oregon, Eugene, Oregon, United States

[5] Fariborz Maseeh College of Engineering and Computer Science, Portland State University, Portland, Oregon, United States

[6] Department of Building Science, Tsinghua University:(清华大学建筑学院), Beijing, China

[7] School of Food and Agriculture, University of Maine, Orono, Maine, United States

Corresponding Author: Suzanne L Ishaq
Email address: sue.ishaq@maine.edu

Di-2-ethylhexyl phthalate (DEHP) is a plasticizer used in consumer products and building materials, including polyvinyl chloride flooring material. DEHP adsorbs from material and leaches into soil, water, or dust, and presents an exposure risk to building occupants by inhalation, ingestion, or absorption. A number of bacterial isolates are demonstrated to degrade DEHP in culture, but bacteria may be susceptible to it as well, thus this study examined the relation of DEHP to bacterial communities in dust. Polyvinyl chloride flooring was seeded with homogenized house dust and incubated for up to 14 days, and bacterial communities in dust were identified at days 1, 7, and 14 using the V3-V4 regions of the bacterial 16S rRNA gene. DEHP concentration in dust increased over time, as expected, and bacterial richness and Shannon diversity were negatively correlated with DEHP concentration. Some sequence variants of *Bacillus*, *Corynebacterium jeddahense*, *Streptococcus*, and *Peptoniphilus* were relatively more abundant at low concentrations of DEHP, while some *Sphingomonas*, *Chryseobacterium*, and a member of the Enterobacteriaceae family were relatively more abundant at higher concentrations. The built environment is known to host lower microbial diversity and biomass than natural environments, and DEHP or other chemicals indoors may contribute to this paucity.

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5 [3,6], Suzanne L. Ishaq[1,7]*

7 Author emails:
8 SV: smv@uoregon.edu
9 CB: chenyangbi@vt.edu
10 JK: jkline@uoregon.edu
11 SN: snunez@uoregon.edu
12 RC: rcorsi@pdx.edu
13 YX: xuying@mail.utexas.edu
14 SLI: sue.ishaq@maine.edu

16 **Affiliations**:
17 [1] Biology and the Built Environment Center, University of Oregon, Eugene, OR, 97403
18 [2] Department of Civil Environmental Engineering, Virginia Polytechnic Institute and State
19 University, Blacksburg, VA 24061 (current)
20 [3] Department of Civil, Architectural and Environmental Engineering, University of Texas,
21 Austin, TX 78712
22 [4] Energy Studies and Buildings Laboratory, University of Oregon, Eugene, OR, 97403
23 [5] Fariborz Maseeh College of Engineering and Computer Science, Portland State University,
24 Portland, OR 97207 (current)
25 [6] Department of Building Science, Tsinghua University, 100084, Beijing, P. R. China (current)
26 [7] School of Food and Agriculture, University of Maine, Orono, ME 04469 (current)

27

28 *** Corresponding author**
29 Suzanne Ishaq, 130A Hitchner Hall, School of Food and Agriculture, University of Maine,
30 Orono, ME 04469. sue.ishaq@maine.edu. 207-581-2770.

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32 **Keywords**: DEHP; gas chromatography; Illumina MiSeq; indoor microbiome; vinyl flooring

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34 **Funding**: This work was funded by grants from the Alfred P. Sloan Foundation to the Biology
35 and the Built Environment Center (BioBE) at the University of Oregon.
36 **Conflict of Interest:** The authors declare no conflict of interest.
37 **Author contributions:** RC, SI, JK, and YX conceived of scope and experimental design, and
38 contributed to writing and editing. CB and SN performed chemical and microbial laboratory
39 protocols, respectively. SV performed DNA sequencing data analysis, literature review, writing,
40 and editing, under the guidance of SI.

41
42 **Abstract**
43
44 Di-2-ethylhexyl phthalate (DEHP) is a plasticizer used in consumer products and building

45 materials, including polyvinyl chloride flooring material. DEHP adsorbs from material and

46 leaches into soil, water, or dust, and presents an exposure risk to building occupants by

47 inhalation, ingestion, or absorption. A number of bacterial isolates are demonstrated to degrade

48 DEHP in culture, but bacteria may be susceptible to it as well, thus this study examined the

49 relation of DEHP to bacterial communities in dust. Polyvinyl chloride flooring was seeded with

50 homogenized house dust and incubated for up to 14 days, and bacterial communities in dust were

51 identified at days 1, 7, and 14 using the V3-V4 regions of the bacterial 16S rRNA gene. DEHP

52 concentration in dust increased over time, as expected, and bacterial richness and Shannon

53 diversity were negatively correlated with DEHP concentration. Some sequence variants of

54 *Bacillus*, *Corynebacterium jeddahense*, *Streptococcus*, and *Peptoniphilus* were relatively more

55 abundant at low concentrations of DEHP, while some *Sphingomonas*, *Chryseobacterium*, and a

56 member of the Enterobacteriaceae family were relatively more abundant at higher

57 concentrations. The built environment is known to host lower microbial diversity and biomass

58 than natural environments, and DEHP or other chemicals indoors may contribute to this paucity.

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70   **Introduction**

71   Di-2-ethylhexyl phthalate (DEHP), a known endocrine-disrupting chemical, is widely

72   used in consumer goods and building materials, from where it is leached into dust, water, or soil

73   and is readily inhaled, absorbed, or ingested by building occupants (Xu et al., 2009; Just et al.,

74   2015; Rowdhwal & Chen, 2018; Bi et al., 2018). DEHP concentration is significantly higher in

75   settled (i.e. surface) dust in homes with polyvinyl flooring (Just et al., 2015; Bi et al., 2018), as

76   well as in HVAC filters (Bi et al., 2018), indicating regular aerial dispersion from floor surfaces.

77   DEHP has been associated with increased reports of children's asthma during warmer seasons

78   (Bi et al., 2018) and body mass index (Wang et al., 2013), and its byproducts or metabolites can

79   likewise be hazardous to human health (Just et al., 2015; Rowdhwal & Chen, 2018; Bi et al.,

80   2018; Bope et al., 2019).

81   Due to the proximity to human occupants and risk for exposure, indoor chemistry is

82   relevant to health (Just et al., 2015; Wang et al., 2017; Rowdhwal & Chen, 2018). But our

83   understanding of indoor chemistry is complicated by the diversity of chemical compounds

84   present in the built environment, emission from materials, chemical interactions (e.g. adsorption,

85   absorption) with materials, and local environmental conditions such as ventilation rate and

86   temperature, all of which can alter chemical reactivity (Bi, Liang & Xu, 2015; Weschler &

87   Carslaw, 2018). Moreover, some monocultures of bacteria may degrade chemical contaminants

88   *in vitro* (Chao & Cheng, 2007; Vamsee-Krishna & Phale, 2008; Liang et al., 2008; Yang et al.,

89   2018), including the phthalates that are emitted from vinyl flooring, as well as in soil or other

90   environmental samples (Liang et al., 2008; Yang et al., 2018; Zhu et al., 2018). However, *in situ*

91   microbial communities in indoor dust have only been demonstrated to degrade chemical

92   contaminants under conditions of high relative humidity (80 -100 %), and this appeared to be

93 largely due to fungal degradation (Bope et al., 2019). It has been suggested that microorganisms

94 naturally produce DEHP, particularly microorganisms in soils, (Ortiz & Sansinenea, 2018).

95 Regardless, the presence of DEHP or other chemicals in dust may affect microbial survival

96 (Brundrett et al., 2018; Lotfy et al., 2018; Zhu et al., 2018), which would alter the composition of

97 microorganisms in dust and potential community dynamics.

98   Little research has been done to elucidate the relationships between phthalate

99 composition and the effects on microbial communities in dust. This pilot study investigated

100 whether dust which accumulated DEHP emitted from polyvinyl chloride flooring would affect

101 the bacterial communities in dust gathered from homes.

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104 **Materials & Methods**

105   Experimental design was similar to previous studies (Wu et al., 2016; Bi et al., 2018).

106 Polyvinyl chloride flooring was cut into 60 mm diameter specimens, placed into glass petri

107 dishes, and sprayed evenly with freshly collected, homogenized house dust containing living

108 microbial communities (Fahimipour et al., 2018) at a concentration of 5 g/m^2. The dishes were

109 placed into a 10 L stainless steel container with one inlet and one outlet for airflow, with an air

110 change rate of 0.5 hr^{-1}, ~0% relative humidity (RH), 25 ± 0.5°C, and no light. Experimental

111 control samples of dust in glass petri dishes, one per time point, were subjected to identical

112 conditions without the inclusion of vinyl flooring, however the homogenized house dust did

113 contain some background DEHP (291 µg/g dust). All samples were randomly placed in the steel

114 container and selected for removal at each time period. During the incubation periods, DEHP

115   was passively adsorbed from polyvinyl flooring and loaded into dust in the experimental

116   samples.

117   Petri dishes were cleaned three times with hexane through ultrasonication and baked at

118   80 °C for 12 hours before experiments. Prior to sample measurements, five matrix spiking

119   experiments were conducted for DEHP; and the recovery efficiencies were between 72% and

120   102%. Additionally, all samples were spiked with three surrogate chemicals (i.e., dibenzyl

121   phthalate, diphenyl isophthalate, and diphenyl phthalate)to determine analytical recovery

122   efficiencies. The average recovery for the surrogates in all samples was 85.3%± 7.3%. Each

123   sample was weighed three times using a five-digit microbalance and mean weight was

124   calculated. To check drift of the GC system, calibration standards were run after every six

125   samples, and results were accepted only when the standards varied less than 10%. A calibration

126   curve, which has a $R^2>0.9999$, was constructed using six different concentrations in the range of

127   1-500 ng/µL for DEHP. Lab blanks (i.e., empty petri dish) were collected and treated identically

128   to the samples (except that no dust was collected). None of the target compounds were detected

129   in these laboratory blanks. To avoid contamination, all glassware and metal ware used for

130   sample collection and analysis were ultrasonically cleaned in methanol for 20 minutes, rinsed

131   with methanol three times, and then baked at 80 °C in an oven prior to use.

132   Following the incubation period, the dust placed on the source material within the petri

133   dish was removed gently with a quantitative filter and transferred to an 8-ml glass vial. The vial

134   was weighed before and after placing the dust to determine the mass of dust collected in each

135   petri dish. The collected dust samples were ultrasonically extracted three times for 30 min each

136   with n-hexane (Hexane (mixture of isomers) ≥98.5% ACS, VWR Chemicals BDH). The volume

137   of the extract was concentrated to approximately 500 µL by gently bubbling the samples with

138   ultrapure nitrogen. DEHP concentrations in the extract was then analyzed using a GC-FID

139   system (Agilent 7890A) equipped with a DB-5ht column (30 m, 0.25 mm, 0.1 μm). The GC-

140   FID system was operated using a splitless injection at 275°C. The temperature program was set

141   at 120°C and held for 2 min, ramped up 12°C/min for 15 min and held 3 min, and then ramped

142   up 20°C/min for 2 min and held 2 min. The detector was set at 320°C. Helium was used as the

143   carrier gas with a constant column flow set at 1.2 ml/min. A calibration standard was run prior

144   to each GC injection, and the variance was always below 10% for all injections.

145   Sterile cotton swabs were used to collect dust from remaining petri dishes for microbial

146   analysis. A dust sample was collected at time point 0, and three experimental replicates were

147   collected each at day 1, 7, and 14, using sterile swabs. A sterile swab and petri dish, and no-

148   template extraction and PCR samples, were included as negative controls. Plates were stored at -

149   20°C until DNA extraction was performed; swab tips were added directly to bead tubes for

150   extraction. Nucleic acids were extracted using the MoBio PowerSoil DNA Extraction Kit

151   (MoBio, Carlsbad, CA, USA) following kit instructions.

152   The V3 and V4 (319F-806R) regions of the 16S rRNA gene were PCR-amplified

153   following a previously described protocol (Kembel et al., 2014), and amplicons were purified

154   with a bead-based clean-up using Mag-Bind RxnPure Plus (Omega Bio-tek, Norcross, GA,

155   USA). Cleaned DNA was quantified using Quant-iT dsDNA assay kit, and pooled with equal

156   concentrations of amplicons for Illumina Miseq ver. 4 paired-end sequencing using a 250-cycle

157   kit at the University of Oregon Genomics Core (Eugene, OR). Raw data are available through

158   the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under

159   BioProject accession PRJNA541585.

160   Sequence data were processed using the DADA2 pipeline ("DADA2 Pipeline Tutorial

161   (1.4)"; Callahan et al., 2016) in R statistical program (R Core Team, 2018). During filtering,

162   sequences were trimmed by 10 bases at the start position, and 10 and 20 bases from the end

163   position for forward and reverse reads, respectively. No ambiguous bases were permitted, and

164   the maximum expected errors (maxEE) were 2 and 3 for forward and reverse, respectively.

165   DNA matching the phiX sequencing controls was removed. Error rates were learned on 1 x 10^6

166   randomly-selected dereplicated reads, and used to identify sequence variants (SVs), after which

167   forward and reverse reads were merged into contiguous sequences. Bimeras (two-parent

168   chimeras) were identified and removed, and taxonomy assigned using the Silva NR ver 132

169   database (Yilmaz et al., 2014). Laboratory negative controls were used to remove identical

170   sequence variants from experimental samples, using adapted code (Ishaq, 2017). Two

171   experimental controls did not successfully generate sequences, and were included in certain

172   figures but removed from the analysis prior to statistical comparison. Samples were rarefied to

173   6,732 sequences per sample, and statistical comparison was performed with R packages phyloseq

174   (McMurdie & Holmes, 2013), vegan (Oksanen, 2018), and rfPermute (Breiman et al., 2015;

175   Archer, 2019).

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179   **Results and Discussion**
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181   Home dust was incubated on polyvinyl chloride flooring, from which the dust was seeded

182   with di-2-ethylhexyl phthalate (DEHP) over 14 days, averaging 1,041 µg/g on day 1, 4,547 µg/g

183   on day 7, and 10,066 µg/g on day 14 (Figure S1).

184   Bacterial communities in dust samples exhibited reduced richness on day 14 (Figure 1A;

185   lm, t = -2.560, p = 0.043, adjusted R^2 = 0.366) and was negatively correlated with DEHP

186   concentration, although significance was only trending (lm, t = -1.939, p = 0.094, adjusted R^2 =

187   0.256). Shannon diversity was not affected by Day or DEHP concentration (Figure 1B; Mann-

188   Whitney, p > 0.05). Control dust, which was incubated on glass plates, exhibited variable

189   richness and diversity, but days 0 and 7 had such low richness (Figure S2) as to be removed

190   during rarefaction. As no replicates were included in microbial analysis it is unclear whether

191   sample days 0 and 7 failed sequencing procedures or accurately had lower bacterial richness.

192   Dust samples clustered significantly by Day (Figure 2; PermANOVA, unweighted

193   Jaccard, f = 1.147, R^2 = 0.141, p = 0.016. A number of bacterial taxa were associated with

194   DEHP concentration (Figure 3). In particular, two *Bacillus* sp., *Corynebacterium jeddahense*, a

195   *Streptococcus* sp., and a *Peptoniphilus* sp. were relatively more abundant at low concentrations,

196   while multiple *Sphingomonas* sp, a *Chryseobacterium* sp., and a member of the

197   Enterobacteriaceae family were relatively more abundant at higher concentrations of DEHP.

198   Several bacterial taxa were significantly associated with control samples with no DEHP (Figure

199   3B), only some of which showed a decline in relative abundance between days 1 and 14.

200   Phylum-level taxonomy for all samples is provided in Figure S3.

201   Bacterial community richness declined over the 14 day study period, and while only

202   trending towards being affected by DEHP, this may reflect low statistical power. And while

203   DEHP concentration is inherently linked to Day, we speculate that the marginal effect observed

204   here is due to SVOC concentration. Bacteria are known to survive in dust even after 90 days

205   with no input of resources, and in particular, when incubated in the dark (Fahimipour et al.,

206   2018). In the present study, only two controls had sufficient data for analysis, but the day 14

207   sample did have higher richness than the control day 1. Additionally, the bacterial taxa which

208   were significantly associated with the controls showed similar relative abundance between the

209   two time points. We posit that the bacterial community in our control samples remained stable

210   during the experiment.

211   However, a more thorough investigation is needed to understand the dynamic here,

212   including additional time points, replication in the controls, and culture assays to determine if

213   bacteria which changed in relative abundance indeed increase or decrease when incubated with

214   DEHP. It is also possible that DEHP itself had no direct effect, but that the degradation or

215   utilization of DEHP by certain taxa created byproducts or competitive inhibitors which affected

216   the microbial community. While preliminary, these results represent the first investigation into

217   the effect of DEHP on an entire bacterial community in dust. Despite the recent scientific

218   advances in indoor chemistry and indoor microbiology, respectively, very little work has been

219   done to elucidate the interaction between chemicals in the built environment and the

220   microorganisms found there.

221   Semi-volatile organic compounds (SVOCs) are commonly incorporated into

222   manufactured products for a variety of purposes, but in building materials they may leach and

223   accumulate in dust on surfaces, adsorb to other materials, or react to form a variety of chemical

224   byproducts (Lucattini et al., 2018; Bi et al., 2018). DEHP readily adsorbs and desorbs from

225   suspended particles, i.e. dust, and can be readily transported this way (Xu et al., 2009).

226   Moreover, in addition to facilitating particle transport, increasing air exchange only increases

227   DEHP emission rates from vinyl flooring (Xu et al., 2009). Thus, DEHP could potentially affect

228   bacterial communities not adjacent to vinyl sources. The built environment is known to host

229   lower microbial diversity and biomass than natural environments (Gibbons, 2016; NASEM,

230   2017), and the presence of DEHP or other SVOCs may be contributing to this paucity.

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322 **Figure Legends**
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325 **Figure 1 Observed richness (SVs) and Shannon Diversity for bacteria in dust, which was**
326 **incubated on polyvinyl chloride flooring and thereby accumulated di-2-ethylhexyl**
327 **phthalate, over 14 days.** Data are rarefied and quality-filtered.
328
329 **Figure 2 Non-metric multidimensional scaling plot of bacterial community membership in**
330 **dust, which was incubated on polyvinyl chloride flooring and thereby accumulated di-2-**
331 **ethylhexyl phthalate, over 14 days.** Lowest stress = 0.067.
332
333 **Figure 3 Bacterial taxa significantly associated with di-2-ethylhexyl phthalate in dust (A),**
334 **compared to controls (B).** Panels are faceted by Day (1, 7, 14). Only significant features are
335 shown (rfpermute, $p < 0.05$).

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339   **Supplemental Material**
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341   **Figure S1 Di-2-ethylhexyl phthalate concentration in experimental samples of dust**
342   **incubated on polyvinyl chloride floor over 14 days.**
343
344   **Figure S2 Observed richness (SVs) and Shannon Diversity for all rarefied, quality-filtered**
345   **sequence data, for bacterial communities in dust, which was incubated on polyvinyl**
346   **chloride flooring and thereby accumulated di-2-ethylhexyl phthalate, over 14 days.**
347
348   **Figure S3 Phylum-level taxonomy of bacterial communities in dust, which was incubated**
349   **on polyvinyl chloride flooring and thereby accumulated di-2-ethylhexyl phthalate, over 14**
350   **days.**

Figure 1

Observed richness (SVs) and Shannon Diversity for bacteria in dust, which was incubated on polyvinyl chloride flooring and thereby accumulated di-2-ethylhexyl phthalate, over 14 days.

Data are rarefied and quality-filtered.

A

B
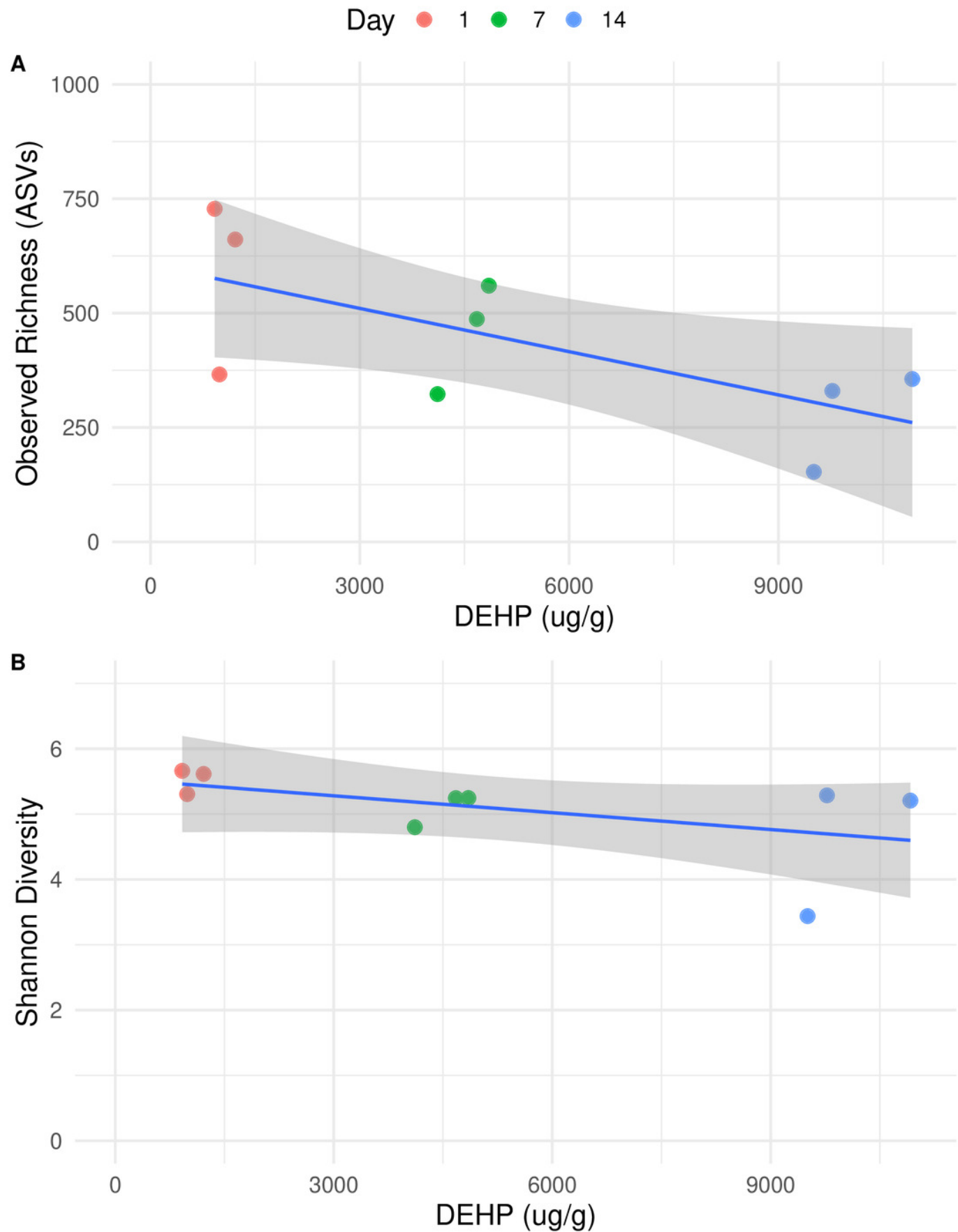
Figure 2

Non-metric multidimensional scaling plot of bacterial community membership in dust, which was incubated on polyvinyl chloride flooring and thereby accumulated di-2-ethylhexyl phthalate, over 14 days.

Lowest stress = 0.067.
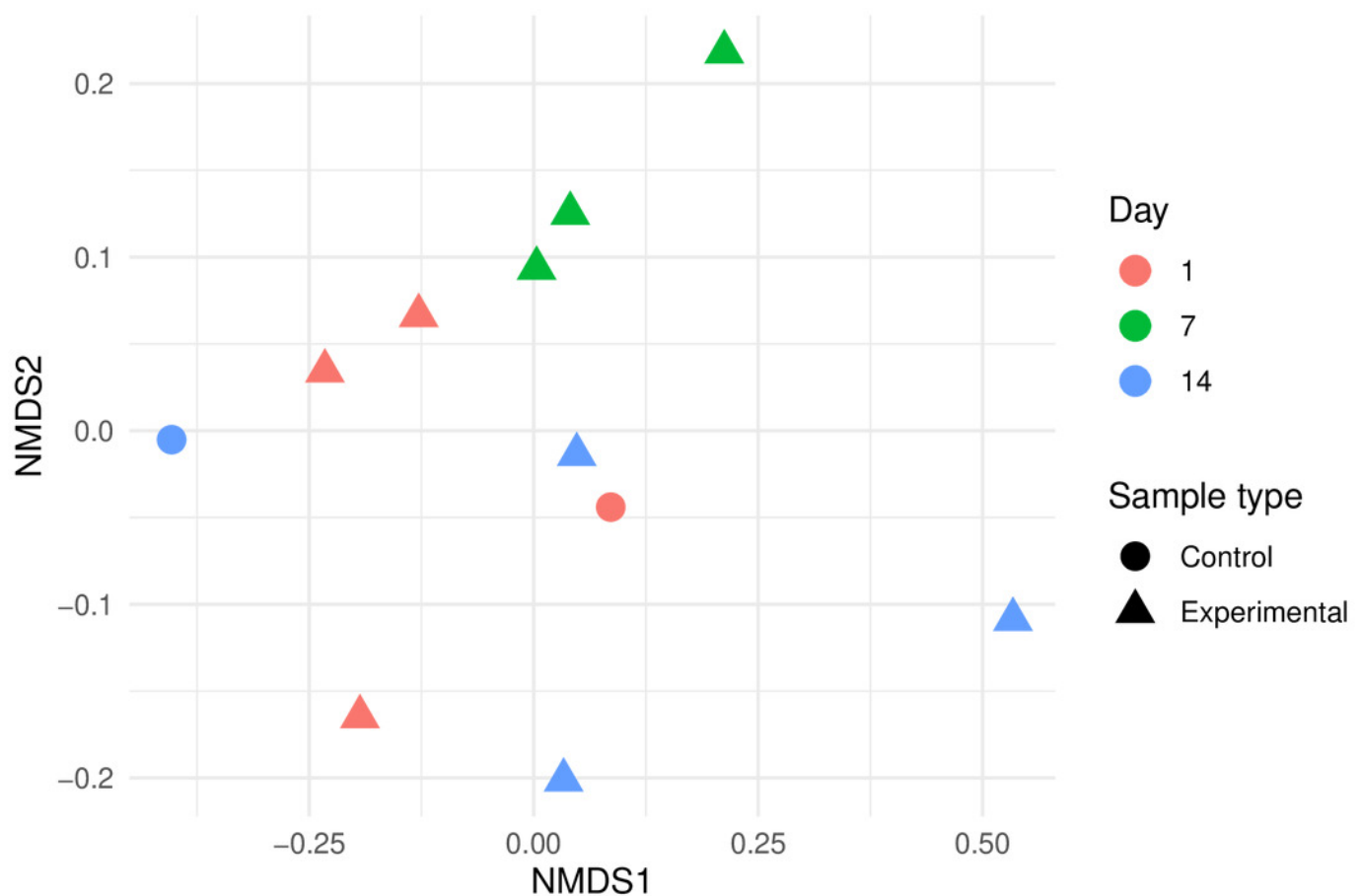
Figure 3

Bacterial taxa significantly associated with di-2-ethylhexyl phthalate in dust (A), compared to controls (B).

Panels are faceted by Day (1, 7, 14). Only significant features are shown (rfpermute, p < 0.05).