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1 Plant diversity enhances the natural attenuation of polycyclic aromatic compounds
2 (PAHs and oxygenated PAHs) in grassland soils

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24

25 ABSTRACT

26 Increasing plant species richness (SR) stimulates microbial activity in soil which might favor
27 biodegradation of polycyclic aromatic compounds (PACs). To explore the relationship between
28 plant community composition and PACs in an urban soil, we determined the concentrations of 29
29 polycyclic aromatic hydrocarbons (PAHs) and 15 oxygenated PAHs (OPAHs) in topsoils from 80
30 plots of a biodiversity experiment. The plots included different SR (1, 2, 4, 8, 16, 60 species) and
31 1-4 plant functional groups (grasses, small herbs, tall herbs, and legumes) in a randomized block
32 design. The concentrations (ng g^{-1}) of $\sum 29\text{PAHs}$ and $\sum 15\text{OPAHs}$ in the soils were 271-2407 and
33 57-329, respectively. Concentrations of 16 (out of 44) PACs and the $\sum 29\text{PAHs}$ were significantly
34 reduced with increasing SR after accounting for the block and soil organic carbon concentration
35 effects (ANCOVA, $p < 0.05$). Microbial turnover as the mechanism underlying this relationship
36 was supported by the findings that (i) the regression slopes of PAHs concentrations >4 rings on
37 SR were negatively correlated with their octanol-water partitioning coefficients, (ii) two OPAHs
38 accumulated in soils with higher SR, and (iii) higher SR increased four OPAH/parent-PAH ratios.
39 We conclude that higher SR can be used to enhance biodegradation of aged PACs in soil. We
40 however caution that OPAHs (some of which are more toxic than their related PAHs) might
41 accumulate in soils during such a plant-assisted remediation process.

42

43 **Keywords:** Plant diversity, Phytoremediation, PAHs, oxygenated PAHs, soils

44 **1. Introduction**

45 Polycyclic aromatic compounds (PACs), such as polycyclic aromatic hydrocarbons (PAHs) and
46 oxygenated PAHs (OPAHs) are ubiquitous in soils. These compounds are mainly released from
47 combustion processes, with additional sources of OPAHs mainly from transformation of PAHs
48 by biological (microbial, enzymatic) and abiotic (photochemical, thermal, photolytic) reactions
49 (Fatiadi, 1967; Keyte et al., 2013; Lundstedt et al., 2007; Wilcke et al., 2014b) . Soils at
50 industrial and urban sites and near roads are frequently contaminated with PACs (Arp et al.,
51 2014; Bandowe et al., 2014a; Bandowe et al., 2011; Choi et al., 2009; Wilcke, 2000). Several
52 representatives of the PACs are toxic (ecotoxic, genotoxic, mutagenic, estrogenic),
53 bioaccumulative, persistent, and therefore cause severe damage to ecosystems, animal and
54 human health (Arp et al., 2014; IARC, 2010; Lundstedt et al., 2007). PACs in soils are dissipated
55 by processes such as biodegradation, plant uptake, bioaccumulation, volatilization, and leaching,
56 that determines their overall residence time and fate (Semple et al., 2003).

57 Plants modulate the concentration of PACs in soil by several processes, particularly by their
58 effect on microbial and enzyme activity and associated turnover of PACs (Collins et al., 2006;
59 Cousins et al., 1999; Desalme et al., 2013; Pilon-Smits, 2005; Wild et al., 2005a, b). The use of
60 plants to clean-up soils contaminated with PACs and other organic pollutants, termed
61 phytoremediation, is a cost-effective and environment-friendly method. The results of intensive
62 research on identifying processes, mechanisms, and useful plants or plant mixtures involved in
63 phytoremediation and natural attenuation of organic pollutants have been summarized in several
64 reviews (Aken et al., 2009; Arthur et al., 2005; Pilon-Smits, 2005). The previous results have
65 shown that some plants such as members of the family of Fabaceae (legumes) and some
66 combinations of several plants can enhance PACs degradation. The limitations of these studies

67 include the fact that experiments were often run with a low number of plant species (partly in a
68 non-full-factorial design), for a limited length of time, and frequently with spiked soils.
69 Moreover, the degradation of primary OPAHs, and the formation and accumulation of OPAHs
70 from PAHs during natural attenuation and/or technical (bio)remediation of PACs-contaminated
71 soils have hardly been considered in phytoremediation experiments (Bamforth and Singleton,
72 2005; Chibwe et al., 2015; Lundstedt et al., 2007; Wilcke et al., 2014b). This is despite the fact
73 that some OPAHs pose comparable (eco)toxicological risks as the PAHs (Dai et al., 2018;
74 Lundstedt et al., 2007; Wincent et al., 2015).

75 In the recent past, the influence of biodiversity on many ecosystem functions has been studied
76 in designed experiments, in which the species richness and other properties of the plant
77 community were systematically manipulated (Spehn et al., 2005; Tilman et al., 1996; Weisser et
78 al., 2017). One of the largest long-term biodiversity experiments is since 2002 the Jena
79 Experiment which varies plant species richness (SR) from 1 to 60 in a randomized block design
80 in a temperate grassland. The Jena Experiment includes the full range of species richness from
81 monocultures which are often used to establish lawns to the natural species richness of a
82 undisturbed meadow (Roscher et al., 2004; Weisser et al., 2017). Furthermore, the Jena
83 Experiment is designed in a way that allows to separate species richness effects *per se* from
84 effects of specific functional groups (legumes, small and tall herbs, and grasses) or mixtures of
85 functional groups. Many of the reported plant diversity effects from the Jena Experiment on
86 microclimatic conditions, water balance, nutrient cycles, microbial abundance and diversity, and
87 enzyme concentrations in soil could directly affect the turnover of PACs in soil (Eisenhauer et
88 al., 2010; Eisenhauer et al., 2011; Oelmann et al., 2011; Rosenkranz et al., 2012; Weisser et al.,
89 2017). Furthermore, the Jena Experiment was established at an urban site on a formerly plowed

90 and thus homogenized arable soil, where we expected a homogeneous PACs contamination
91 because of atmosphere-soil partitioning between a well-mixed topsoil and a homogeneous urban
92 atmosphere only driven by small-scale variations in soil organic carbon (SOC) concentrations.
93 The Jena Experiment therefore offers a unique opportunity to gain mechanistic insights in the
94 role of plant diversity on PACs concentrations in soil.

95 The objectives of this study were to investigate - to our knowledge for the first time - if plant
96 diversity (SR, functional group richness, and presence of specific functional groups) (i)
97 influences PACs concentrations in grassland soil and (ii) by which driving processes the plant
98 diversity-PACs concentrations relationship can be explained. We hypothesize that concentrations
99 of most PAHs and primary OPAHs decrease with increasing SR and plant functional group
100 richness and in the presence of individual plant functional groups because of enhanced
101 dissipation, while secondary OPAHs are formed as degradation products. We furthermore
102 hypothesize that the reason for the changes in PACs concentrations are mainly attributable to
103 microbial degradation.

104

105 **2. Experimental section**

106 **2.1. Study site and experimental design**

107 This study was conducted as part of the Jena Experiment (www.the-jena-experiment.de), which is a
108 grassland plant diversity experiment addressing the role of plant diversity for element cycling
109 and trophic interactions (Roscher et al., 2004). The Jena Experiment is located in the city of Jena,
110 Germany (50°55'N, 11°35'E; 130 m above sea level, ca. 110.000 habitants) on the floodplain of
111 the Saale River. Mean annual air temperature is 9.9 °C, and mean annual precipitation is 610 mm
112 (1980–2010) (Hoffmann et al., 2014). The soil is an Eutric Fluvisol that developed from up to 2-

113 m thick loamy fluvial sediments, almost free of stones (IUSS Working Group, 2014). As a result
114 of the fluvial dynamics, the texture ranges from sandy loam near the river to silty clay with
115 increasing distance from the river. This systematic variation in soil texture is considered in the
116 experimental design as the plots are arranged in four blocks that are parallel to the river Saale
117 and each has approximately homogeneous soil texture. The site was converted from grassland to
118 an arable field in the 1960s and thereafter fertilized and plowed for crop production until the
119 beginning of the grassland plant diversity experiment in 2002 (Roscher et al., 2004).

120 The entire experimental design is has been described previously (Roscher et al., 2004).
121 Briefly, the main experiment comprises 82 plots (20 m × 20 m) grouped in four blocks
122 (considering the systematic variation in soil texture). The 82 plots were established from seeds in
123 May 2002 with different levels of species richness (1, 2, 4, 8, 16, or 60 plant species) and plant
124 functional group richness (1-4 plant functional groups out of grasses, small herbs, tall herbs, and
125 legumes) chosen by the random replacement method. The species were selected from a pool of
126 60 species frequently occurring in nutrient-rich agricultural grasslands (*Arrhenatherion*
127 meadows) (Ellenberg and Leuschner, 2010). Each level of species richness is replicated four
128 times per block, resulting in 16 plots per richness level except for the 60-species plots, which
129 were replicated once per block, i.e. four times in the whole experiment. There was a strong
130 correlation between the number of sown species and the realized species richness ($R^2 > 0.9$ in
131 each year during 2003–2007) (Marquard et al., 2009) corroborating the successful establishment
132 of the species richness gradient. The management was adapted to meadows that are managed
133 with low intensity and used for hay production by mowing twice a year in June and September.
134 The plots were regularly weeded to maintain the sown species composition. During the
135 experimental period, the plots were not fertilized. Two plots with monocultures had to be

136 abandoned (*Cynosurus cristatus* L. and *Bellis perennis* L.), resulting in 80 investigated plots in
137 this study.

138

139 **2.2. Sampling and chemical analysis**

140 Soil samples were taken in April 2011, i.e. 9 years after the establishment of the grassland, on
141 each of the 80 plots with stainless-steel corers from the 0-0.05 m soil depth layer. Samples were
142 air-dried and sieved (< 2 mm). An aliquot of each soil sample was milled and their total carbon
143 (TC) concentrations were determined with an elemental analyzer (vario EL cube, Elementar
144 Analysensysteme GmbH, Hanau, Germany). The inorganic C (IC) concentration of each sample
145 was also determined from an aliquot of soil after combusting soil organic carbon (SOC) in a
146 muffle oven (550°C, 2 h). The SOC concentrations were quantified as the difference between the
147 TC and IC.

148 The concentrations of 44 PACs (29 PAHs, 15 OPAHs) in the soil from Jena, European
149 reference materials (ERM-CC013a), and procedural blanks (inert sorbent, Isolute HM-N,
150 Biotage, Sweden) were determined using previously published methods (Bandowe et al., 2014a;
151 Bandowe et al., 2010; Bandowe et al., 2011; Bandowe and Wilcke, 2010; Lundstedt et al., 2014).
152 In brief, soils (11-13 g) were mixed with Isolute HM-N and transferred into 33-mL ASE
153 extraction cells. Each sample was spiked with 50 µL of each of a mixture of 11 deuterated PAHs
154 (10 µg mL⁻¹ of each) and 2 deuterated OPAHs (20 µg mL⁻¹ each). Each sample was then
155 extracted twice by pressurized liquid extraction with an accelerated solvent extractor (ASE, 200).
156 Dichloromethane was used for the first extraction followed by acetone:
157 dichloromethane:trifluoroacetic acid (1%) [250: 125: 1 v/v/v] for the second extraction. The
158 instrumental conditions for the ASE were the same as in previous work (Bandowe and Wilcke,

159 2010). The two extracts from each sample were combined, passed through Na₂SO₄, spiked with
160 hexane and rotary evaporated until < 1 mL remained. Extracts were transferred to a 3-g silica gel
161 (10% deactivated) in a 6-mL glass column. Each sample was eluted with (a) 15 mL hexane:
162 dichloromethane (5:1 v/v) and (b) 8 mL dichloromethane followed by 5 mL acetone. Fractions a
163 and b, which contain PAHs and OPAHs, respectively, were collected in separate flasks. Each
164 flask was spiked with about 0.75 mL of toluene, and then rotary evaporated to < 1 mL before
165 being transferred to 2-mL vials to determine the concentration of target PACs. PAHs and
166 OPAHs were determined in two different runs with a gas chromatograph-mass spectrometer
167 (Agilent 7890 A GC coupled to Agilent 5975 C mass spectrometer). The GC-MS was operated
168 in the electron ionization mode with selected ion monitoring of target PACs. As a check of the
169 accuracy of our analytical procedure, we simultaneously analyzed aliquots (n = 2) of the
170 European reference material (ERM-CCO13a: Polycyclic Aromatic Hydrocarbons in Soil) from
171 the Federal Institute of Materials Research and Testing (BAM), Berlin Germany. Procedural
172 blanks (inert bulk sorbent: Isolute HM-N, n = 3) were also extracted and analyzed with the same
173 methods as samples and reference materials. All data recording and setting up of calibration
174 functions were done with Agilent ChemStation software. Concentrations of target compounds
175 were determined by the internal standard procedure. The average mass of target compounds in
176 blanks was deducted from that in the samples before calculating the final concentrations per dry
177 mass of extracted soil. Further details of quality control procedures are specified in previous
178 papers (Bandowe et al., 2010; Bandowe et al., 2011; Bandowe and Wilcke, 2010). Table S1 lists
179 the names and abbreviations of all analyzed PACs. Results of our quality control procedures are
180 reported in the Supplementary Information.

181

182 **2.3. Calculations and statistical analysis**

183 The sum of the concentrations of all analyzed PAHs is referred to as $\Sigma 29$ PAHs, 16 US-EPA
184 PAHs as Σ US-EPA PAHs, non-alkylated PAHs with 2 to 3 benzene rings as Σ LMW-PAHs, non-
185 alkylated PAHs with 4-7 benzene rings as Σ HMW-PAHs, and OPAHs as $\Sigma 15$ OPAHs. The ratios
186 of the concentration of individual OPAHs and their related parent-PAHs were also calculated, if
187 the OPAH was a known transformation product of the PAH to which it was referred (Bamforth
188 and Singleton, 2005; Casellas et al., 1997; Cerniglia, 1993; Mahajan et al., 1994; Schocken and
189 Gibson, 1984). The concentrations of individual PACs, which were below detection limits and
190 OPAH/parent-PAH concentration ratios, which could not be calculated in soils of 40 or more
191 plots, were excluded from the statistical analysis (i.e., 1,4-NQ, 1,8-NAA, 1,4-NQ/NAPH, 1,8-
192 NAA/ACENY).

193 A hierarchical ANCOVA approach was adopted to detect the influence of plant community
194 composition on PACs concentrations (individual and sums) and OPAH/parent-PAH
195 concentration ratios on the plots. After the establishment of the various plant mixtures, the soil
196 organic matter and PACs concentrations changed depending on the plant mixture on a specific
197 plot. Furthermore, while the whole experimental plot was still in contact with a homogeneous
198 urban atmosphere, the receptor properties of the surface changed in relation with the plant
199 mixture on a specific plot and its effects on soil properties. Our analysis aimed at detecting the
200 effects of the various plant mixtures on PACs concentrations nine years after establishment of
201 the grassland, while securing homogeneous start conditions by eliminating the influence of the
202 initial variation in soil organic matter concentrations. In a first ANCOVA, we therefore included
203 (after the block effect and the 2002-SOC concentrations to eliminate the variation in PACs
204 concentrations driven by the initial soil heterogeneity), SR (log-transformed; covariate) followed

205 by functional group richness (covariate). In a second ANCOVA, we analyzed the influence of
206 the presence or absence of specific plant functional groups by including the explanatory
207 variables in the following order: block, 2002-SOC, species richness (log-transformed; covariate),
208 presence/absence of legumes, grasses, tall herbs, and small herbs (factors). The order of the
209 explanatory variables follows the statistical design of the experiment and the expected strength
210 of the variable's effect because legumes usually have the strongest effect on ecosystem
211 functioning, followed by grasses, and small herbs usually modify ecosystem functions the least.
212 To meet the requirements of the ANCOVA, the data had to be transformed with a Box-Cox
213 power transformation (Equation 1) to render the residuals as close to normal distribution as
214 possible (R function *powerTransform()* from package *car*) (Fox and Weisberg, 2011).

$$215 \quad y'_\lambda = \begin{cases} \frac{(y+1)^\lambda - 1}{\lambda}, \lambda \neq 0 \\ \log(y + 1), \lambda = 0 \end{cases} \quad (\text{Equation 1})$$

216 The hierarchical ANCOVA was calculated with the function *aov()*. Pearson's correlation was
217 calculated with the function *cor.test()*. All statistical analyses were performed with the R 3.2.0
218 software package (R Core Team, 2015).

219 Octanol water partition coefficients (K_{ow}) for PAHs were taken from two sources (Mackay et
220 al., 2006; Neff et al., 2005), while those of the OPAHs were estimated with KOW^{WIN} v1.67 EPI
221 SuiteTM version 4.11 (US EPA: <http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm>).

222

223 **2. Results**

224 At the time when the Jena Experiment was established, SOC concentrations ranged from 14 to
225 28 g kg⁻¹ (mean: 19 g kg⁻¹) and were unrelated to the later established SR, confirming that there
226 was no a-priori bias of the experimental design (Fig. S1a). The SOC concentrations in 2011 (the

227 year of our sampling campaign) were higher than in 2002, ranging from 15 – 34 g kg⁻¹ (mean: 25
228 g kg⁻¹), because of the transformation from a plowed arable soil depleted in organic matter to a
229 permanent grassland (Lange et al., 2015b). The accumulation of soil organic matter was
230 accelerated by increasing plant species richness (Lange et al., 2015a). Therefore, a positive
231 correlation between species richness and SOC concentrations appeared in the year 2011 (Fig.
232 S1b). The SOC concentrations in 2002 (Lange et al., 2015b) and 2011 still correlated (Fig. S6),
233 so that the spatial distribution of SOC concentrations in soil did not change fundamentally. The
234 SOC concentrations in 2002 correlated more closely with those of the Σ 29PAHs and Σ 15OPAHs
235 than the SOC concentrations in 2011 ($r = 0.44$ and 0.53 vs. 0.22 and 0.32 , respectively and $p <$
236 0.05 in all cases, Fig. S2).

237 The concentrations of Σ 29PAHs, Σ US-EPA PAHs and B(A)P averaged 825 ng g⁻¹ (range: 260
238 – 2400), 677 ng g⁻¹ (211 – 2048) and 42 ng g⁻¹ (11 – 140), respectively. The average
239 concentrations of the Σ US-EPA PAHs in soils of the Jena Experiment were higher than the
240 median concentration of 194 ng g⁻¹ reported for rural grassland soils but lower than the median
241 concentration of 1100 ng g⁻¹ in urban areas (Wilcke, 2000). The proximity of the experimental
242 site to roads and the city of Jena may explain the elevated PAH concentrations relative to rural
243 grasslands (Choi et al., 2009; Wilcke, 2000). The PAH mixtures were on average dominated by
244 B(BJK), PYR, PHEN, and FLUA (Figure S3), which is typical of temperate soils in
245 industrialized countries (Wilcke, 2000, 2007). The dominance of HMW-PAHs is revealed by the
246 Σ HMW/ Σ LMW-PAHs ratio which averaged 5.5 (3 – 8). The composition pattern of the PAH
247 mixtures in the Jena soils are typical of emissions from the combustion of fossil fuel (Bandowe
248 et al., 2011; Wilcke, 2000; Wilcke et al., 2014b).

249 The concentrations of $\Sigma 15$ OPAHs averaged 142 ng g^{-1} (range: 57-405). The OPAH mixtures
250 were dominated by 9,10-ANQ, B(A)FLUone, and 9-FLO (Figure S3) which were similarly
251 revealed to be amongst the dominating OPAHs in soils, street dust, and air samples from other
252 urban, traffic, and industrial sites (Bandowe et al., 2014a; Bandowe et al., 2014b; Bandowe and
253 Nkansah, 2016; Bandowe et al., 2011; Wei et al., 2015a; Wei et al., 2015b; Wilcke et al., 2014b).
254 This can be explained by the fact that the OPAHs in the soil are displaying the fingerprint of
255 traffic and urban diffuse sources.

256 Our ANCOVA analysis revealed that after consideration of the block effect and SOC
257 concentrations in 2002 to eliminate soil heterogeneity at the start of the experiment, higher SR
258 significantly decreased the concentrations of 16 PAHs (including the most abundant compounds
259 B(BJK) and PYR), the $\Sigma 29$ PAHs, Σ US-EPA PAHs, Σ LMW-PAHs, and Σ HMW-PAHs (Fig. 1,
260 Tables 1 and S2). Similarly, there was a negative relationship between SR and the concentrations
261 of B(A)FLUone (the second most abundant OPAH, Fig. 2c), 7,12-B(A)A (Fig. 2d), and 5,12-
262 NACQ (Table S3). In contrast, increasing SR led to significant increases in the concentrations of
263 1-NLD and 1,2-ACQ (Fig. 2a,b). Furthermore, increasing species richness resulted in significant
264 increases in the compound concentration ratios 1-INDA/FLUO, 2-BPCD/PHEN, 1,2-
265 ACQ/ACENY, and 1,2-ACQ/ACEN (Fig. 3). There was a negative relationship between the
266 octanol-water partitioning coefficient (K_{OW}) and the slope of the regression line of the
267 concentrations of PAHs with four or more aromatic rings on species richness, while PAHs with
268 less than four rings did not show this relationship (Fig. 4a). For the OPAHs, we found partly
269 positive and partly negative slopes of the regressions on K_{OW} values (Fig. 4b). The slopes tended
270 to be steeper with increasing K_{OW} value, albeit not significantly.

271 Increasing the number of plant functional groups significantly increased the concentration of
272 1-NLD and the 1-NLD/1-MNAPH ratio (Table 1). The presence of legumes resulted in
273 significant increases in the concentration of BP and decreases in the 7, 12-B(A)A/B(A)A ratio
274 (Fig. S4). The presence of grasses significantly reduced the concentration of NAPH, but
275 increased the concentration of 1-NLD and the 1-NLD/1-MNAPH ratio (Fig. S5).

276

277 **4. Discussion**

278 In 2011, SOC concentrations increased with increasing species richness. This should result in
279 increasing PACs concentrations in soil with increasing SR, because under equilibrium
280 conditions, there is a higher partition of PACs from the atmosphere into soils with a higher SOC
281 concentration (Wilcke and Amelung, 2000; Wilcke et al., 2014a). However, the reverse was
282 observed at our study sites (Table 1, Fig. 1). We therefore assume that the additional atmospheric
283 input of PACs with increasing SR and the possibly decreased biodegradation because of stronger
284 sorption of PACs were minor. The stronger correlation between the SOC concentrations and
285 those of $\Sigma 29$ PAHs and $\Sigma 15$ OPAHs in 2002 than in 2011 (Fig. S2) confirmed our assumption that
286 the initial spatial PACs distribution in 2002 was to a large part driven by the SOC
287 concentrations, while the further development of PACs concentrations after the establishment of
288 the grassland was more complex and the influence of SOC concentrations therefore less clear.
289 The consistently negative influence of higher SR on PAH concentrations (Table 1, Fig. 1)
290 indicates increased dissipation of PAHs with increasing SR. Plant uptake, particularly via the
291 roots is one possible process of PAH loss from the soil, but can be considered as minor and
292 should anyway mainly affect the LMW-PAHs (Collins et al., 2006). Enhanced dissipation of
293 PAHs can be related to enhanced volatilization, leaching and/or microbial degradation. Enhanced

294 volatilization would only affect the low molecular weight compounds because the high
295 molecular weight PAHs are little volatile (Cousins et al., 1999; Wang et al., 2015). However, we
296 did not observe a different effect on LMW and HMW compounds (Table 1, Fig. 1) and therefore
297 consider enhanced volatilization with increasing SR as insignificant. Enhanced leaching with
298 increasing SR is possible, because it has been shown that after an initial establishment phase of
299 the grassland from a previous arable field, increasing SR is related with improved water
300 infiltration rates (Fischer et al., 2015). Consequently, the LMW-PAHs could be more strongly
301 leached in dissolved form and the HMW-PAHs bound to dissolved organic matter in the species-
302 rich mixtures. However, leaching favors the LMW compounds (Wilcke, 2000), which should
303 result in a stronger effect of SR on LMW-PAHs, which was not observed possibly because
304 stronger leaching was compensated by stronger sorption because of the higher SOC
305 concentrations with increasing species richness. Consequently, leaching is not a probable
306 explanation of the decreasing PAHs concentrations with increasing SR.

307 It has been reported from our experimental site that increasing SR enhances microbial activity
308 since the fifth vegetation period (i.e. since the year 2006) (Eisenhauer et al., 2010; Eisenhauer et
309 al., 2011). Increasing microbial activity likely results in enhanced biodegradation of PACs. The
310 latter occurs to a small degree metabolically (only low molecular weight compounds) but mostly
311 co-metabolically (Bamforth and Singleton, 2005; Cerniglia, 1993; Peng et al., 2008). At the Jena
312 experimental site, increasing SR leads to enhanced input of root-derived exudates into the soil
313 (Lange et al., 2015a; Lange et al., 2014). The enhanced input of root exudates can specifically
314 stimulate PAHs degradation, beyond the general stimulation of microbial activity, because root
315 exudates can improve the bioavailability of aged PACs (through desorption), with the overall
316 consequence of enhanced degradation of PACs in soils (Aken et al., 2009; Alkorta and Garbisu,

317 2001; Cébron et al., 2011; Gao et al., 2011; Pilon-Smits, 2005). The assumption of a more
318 pronounced degradation of PAHs with increasing SR is further supported by the finding that the
319 topsoil under species-rich mixtures is moister than under species-poor ones (Rosenkranz et al.,
320 2012) (which favors increased microbial activity and thus PAH degradation).

321 Enhanced biodegradation is supported by our findings of increasing OPAH/parent-PAH ratios
322 (Fig. 3) and by the negative relationship between the K_{ow} values and the slopes of the regression
323 lines of individual PAH concentrations on SR (Fig. 4a) for PAHs with four or more rings. The
324 fact that the 2-3 ring PAHs did not fit into the line shown in Fig. 4, might be attributable to the
325 particularly high susceptibility of these compounds to leaching, plant uptake, and more dynamic
326 air-soil exchanges (as a result of rapid changes in the air-soil fugacity ratios) which might
327 confound a degradation effect (Cabrerizo et al., 2011). The positive slopes (for a number of
328 OPAHs) which indicate the formation of these OPAHs as metabolic products of PAHs support
329 our interpretation of microbial PACs turnover as main explanation of the relationship between
330 SR and PACs concentrations in soil (Fig. 4b). The negative slope of (mainly) HMW-OPAHs
331 instead suggest that these compounds are deposited to soil from combustion sources as primary
332 OPAHs or formed in the atmosphere from post-emission transformation of PAHs as secondary
333 OPAHs. Their extent of accumulation or persistence in the soil is similar to those of the HMW-
334 PAHs. We conclude that enhanced microbial degradation of PAHs with increasing species
335 richness is the most probable explanation of our findings.

336 Higher species richness favored the accumulation of particularly LMW- OPAHs including 1-
337 NLD and 1, 2-ACQ but decreased the concentrations of some high HMW-OPAHs such as
338 B(A)FLUone and 7,12-B(A)A (Fig. 2 and 4b). We interpret this finding as an indication of the
339 production of LMW-OPAHs as metabolites from the degradation of PAHs and the dominance of

340 the degradation of HMW-OPAHs after being deposited to our study soil. 1-NLD has been
341 isolated as a product formed from the microbial degradation of 1-MNAPH (Mahajan et al.,
342 1994)[39]. 1,2-ACQ is a metabolic product from the microbial degradation of ACENY and
343 ACEN (Schocken and Gibson, 1984) and was also reported to be formed from PAHs in soils
344 after 19-weeks incubation (Wilcke et al., 2014b). Both, B(A)FLUone and 7,12-B(A)A belonged
345 to the compounds that were predominantly degraded in the study of Wilcke et al., 2014b. We
346 conclude that increasing SR and the associated enhanced biodegradation of PAHs results in the
347 net accumulation of some LMW-OPAHs while the HMW-OPAHs are increasingly degraded like
348 PAHs.

349 There were only few effects of functional group richness, and presence of legumes or grasses
350 (Table 1, Figs. S4, S5). The observation that plant functional group richness has little effect on
351 PACs in soils is in line with the results of a recent study (Ai et al., 2018). Functional group
352 richness and presence of grasses seemed to favor the accumulation of 1-NLD which might
353 indicate its enhanced formation from the biodegradation of 1-MNAPH. Our interpretation is
354 further supported by the increased 1-NLD/1-MNAPH ratios. NAPH was the only PAC with
355 lower concentrations in the presence of grasses. The weak effect of grasses on PACs
356 concentrations was unexpected, because several previous experiments have shown that grasses
357 are capable of enhancing the removal of PAHs from soil through increased microbial activity
358 around their rhizosphere, secretion of exudates that improve bioavailability, and their extensive
359 root morphology (Aprill and Sims, 1990; Binet et al., 2000; Dzantor et al., 2000; Günther et al.,
360 1996; Lee et al., 2008; Phillips, 2008). Binet et al., 2000 studied the degradation of PAHs in the
361 rhizosphere of ryegrass and detected the formation of the OPAHs (9,10-ANQ, 7,12-B(A)A) from
362 the degradation of PAHs. The increase in BP concentrations in the presence of legumes might be

363 explained by several processes including i. their release by legumes, ii. their deposition in the
364 presence of legumes, and iii. their decreased dissipation in the presence of legumes. The positive
365 effect of legumes on the 7,12-B(A)A/B(A)A ratio either suggests that 7,12-B(A)A is also
366 produced by legumes or that the B(A)A concentrations are specifically decreased. Legumes have
367 also been previously reported to favor phytoremediation of PAHs (Dzantor et al., 2000; Lee et
368 al., 2008; Phillips, 2008).

369

370 **5. Conclusions**

371 Our study demonstrates that increasing species richness results in decreasing
372 concentrations of PAHs and HMW-OPAHs in soils of a grassland. Consequently, species-rich
373 grassland mixtures can favor the natural attenuation of PACs-contaminated urban soils. This can
374 be applied in remediation efforts. However, we also demonstrate that some low molecular weight
375 OPAHs accumulate in soil during the degradation of PAHs. This might increase ecotoxicological
376 risks associated with these compounds and therefore requires thorough monitoring during
377 phytoremediation.

378

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409 Table 1. Overview of PACs concentrations and OPAH/parent-PAH concentration ratios that are
 410 significantly influenced by species richness, functional group richness, presence of legumes, and
 411 presence of grasses. Positively affected compound concentrations are underlined and in italic
 412 letters. All other compound concentrations were negatively affected. Detailed statistical results are
 413 shown in Tables S2 and S3.

414

	PAHs	OPAHs	OPAH/parent-PAH ratio
Species richness (log-transformed)	NAPH, ACENY, FLUA, PYR, B(A)A, CHRY, B(BJK), B(E)P, PERY, IND, DIBE, B(GHI), COR, Σ29PAHs, ΣUS-EPA PAHs, ΣLMW-PAHs, ΣHMW-PAHs	<i><u>1-NLD</u></i> , <i><u>1,2-ACQ</u></i> , B(A)FLUone, 7,12-B(A)A, 5,12-NACQ	<i><u>1-INDA/FLUO</u></i> , <i><u>2-BPCD/PHEN</u></i> , <i><u>1,2-ACQ/ACENY</u></i> , <i><u>1,2-ACQ/ACEN</u></i>
Functional group richness	-	<i><u>1-NLD</u></i>	<i><u>1-NLD/1-MNAPH</u></i>
Presence of legumes	<i><u>BP</u></i>	-	7,12-B(A)A/B(A)A
Presence of grasses	NAPH	<i><u>1-NLD</u></i>	<i><u>1-NLD/1-MNAPH</u></i>

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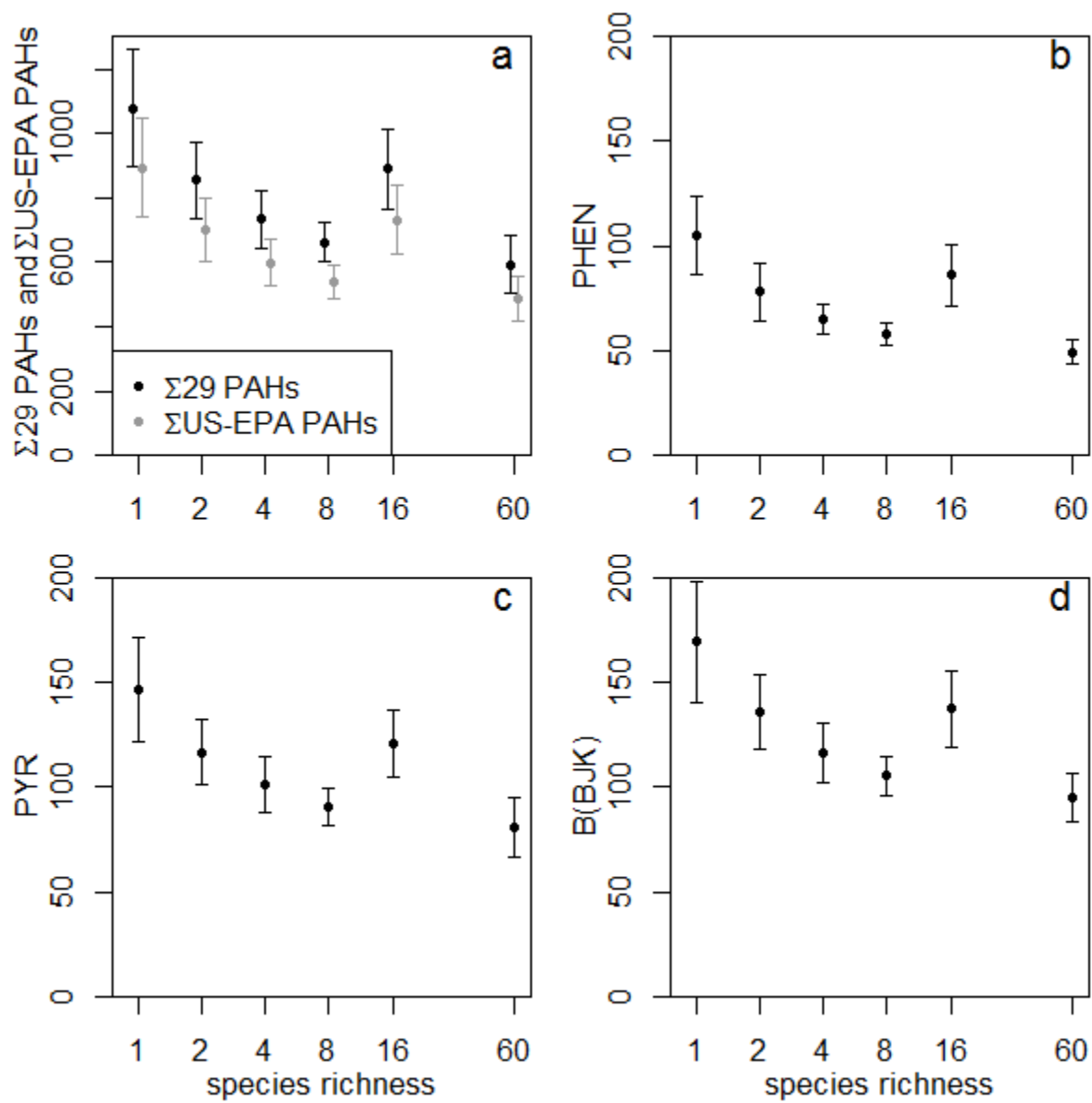
420 **Figure 1.** Relationship between log-transformed species richness and concentrations in ng g^{-1} of
421 a) $\sum 29$ PAHs, (black; ANCOVA: $F = 5.23$, $p = 0.025$) and \sum US-EPA PAHs (gray; $F = 5.13$, $p =$
422 0.026), b) phenanthrene (PHEN; $F = 3.96$, $p = 0.050$), the third most abundant individual PAH
423 (cf. Fig. S3), c) pyrene (PYR; $F = 4.65$, $p = 0.034$), the second most abundant individual PAH
424 (cf. Fig. S3) and d) the benzo[b+j+k]fluoranthenes (B(BJK); $F = 5.30$, $p = 0.024$), the most
425 abundant PAHs. The points display means and the whiskers the standard error.

426 **Figure 2.** Relationship between log-transformed species richness and concentrations in ng g^{-1} of
427 a) 1-naphthaldehyde (1-NLD; ANCOVA: $F = 4.95$, $p = 0.030$), b) 1,2-acenaphthenequinone
428 (1,2-ACQ; $F = 6.78$, $p = 0.011$) and c) benzo[a]fluorenone (B(A)FLUone; $F = 4.31$, $p = 0.041$),
429 and d) benzo[a]anthracene-7,12-dione (7,12-B(A)A; $F = 4.25$, $p = 0.043$). The points display
430 means and the whiskers the standard error.

431 **Figure 3.** Relationship between log-transformed species richness and (unitless) OPAH/parent
432 PAH concentration ratios of a) 1-indanone (1-INDA)/fluorene (FLUO; ANCOVA: $F = 4.84$, $p =$
433 0.031), b) 2-biphenylcarboxaldehyde (2-BPCD)/phenanthrene (PHEN; $F = 6.02$, $p = 0.016$), c)
434 1,2-acenaphthenequinone (1,2-ACQ)/acenaphthylene (ACENY; $F = 13.12$, $p = 0.001$), and d)
435 1,2-acenaphthenequinone (1,2-ACQ)/acenaphthene (ACEN; $F = 9.11$, $p = 0.004$). The points
436 display means and the whiskers the standard error.

437 **Figure 4.** Relationship between log-transformed octanol-water-partitioning coefficients (K_{OW})
438 and the slopes of regression lines of the concentrations of a) PAHs on log-transformed species
439 richness (the red triangles show the PAHs with <4 rings and blue dots the PAHs >4 rings) and b)
440 OPAHs. The dotted regression line in b) illustrates a non-significant tendency. K_{OW} values of
441 PAHs were taken from two sources (Mackay et al., 2006; Neff et al., 2005), while those of the
442 OPAHs were estimated with $K_{OW}WINv1.67EPI$ SuiteTM version 4.11 (US EPA:
443 <http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm>). Chrysene+Triphenylene (CHRY)
444 and the B(BJK) were omitted because we were unable to separate the individual compounds with
445 our analytical method and therefore could not assign K_{OW} values for these compound mixtures.

Figure 1



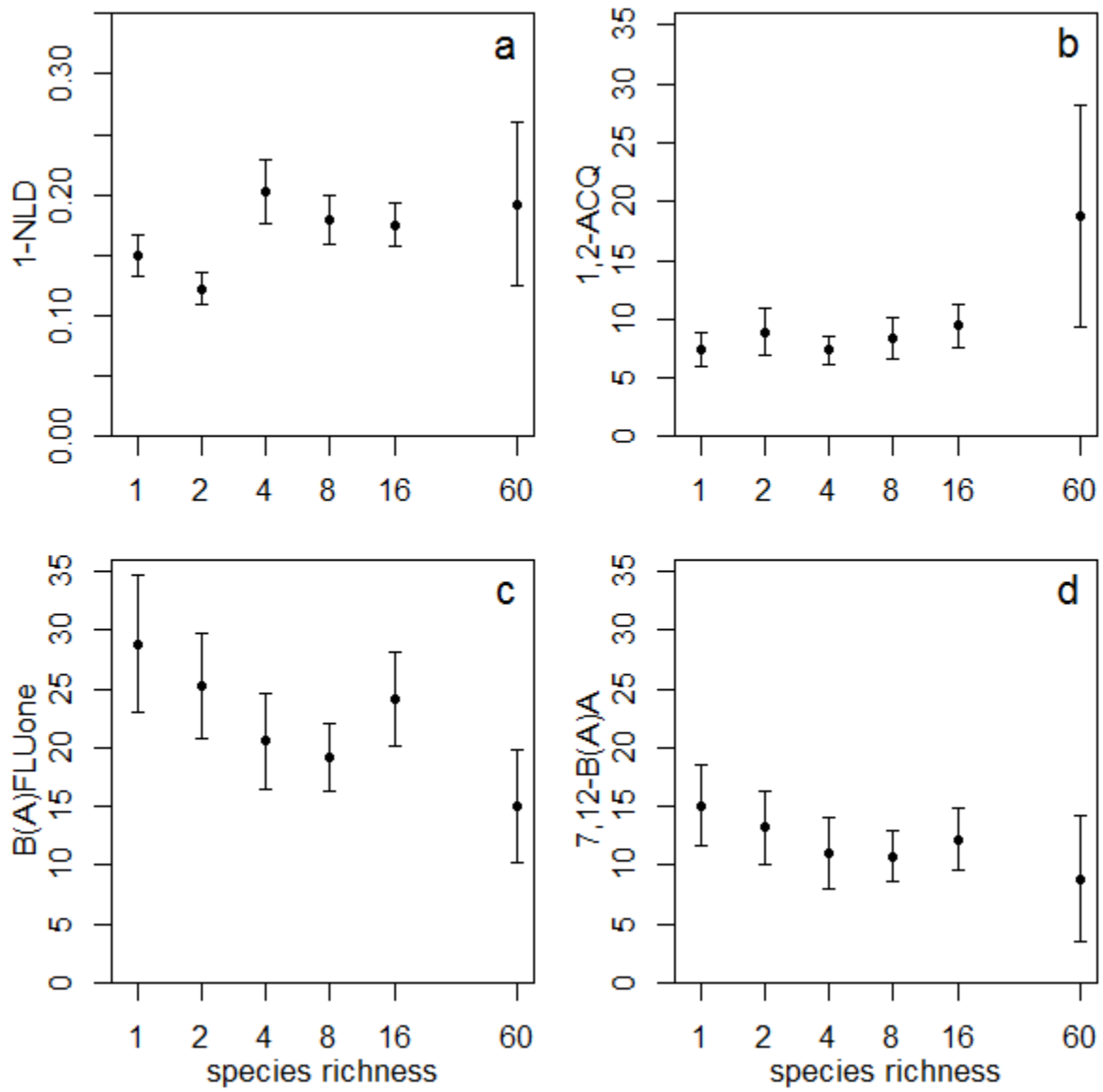
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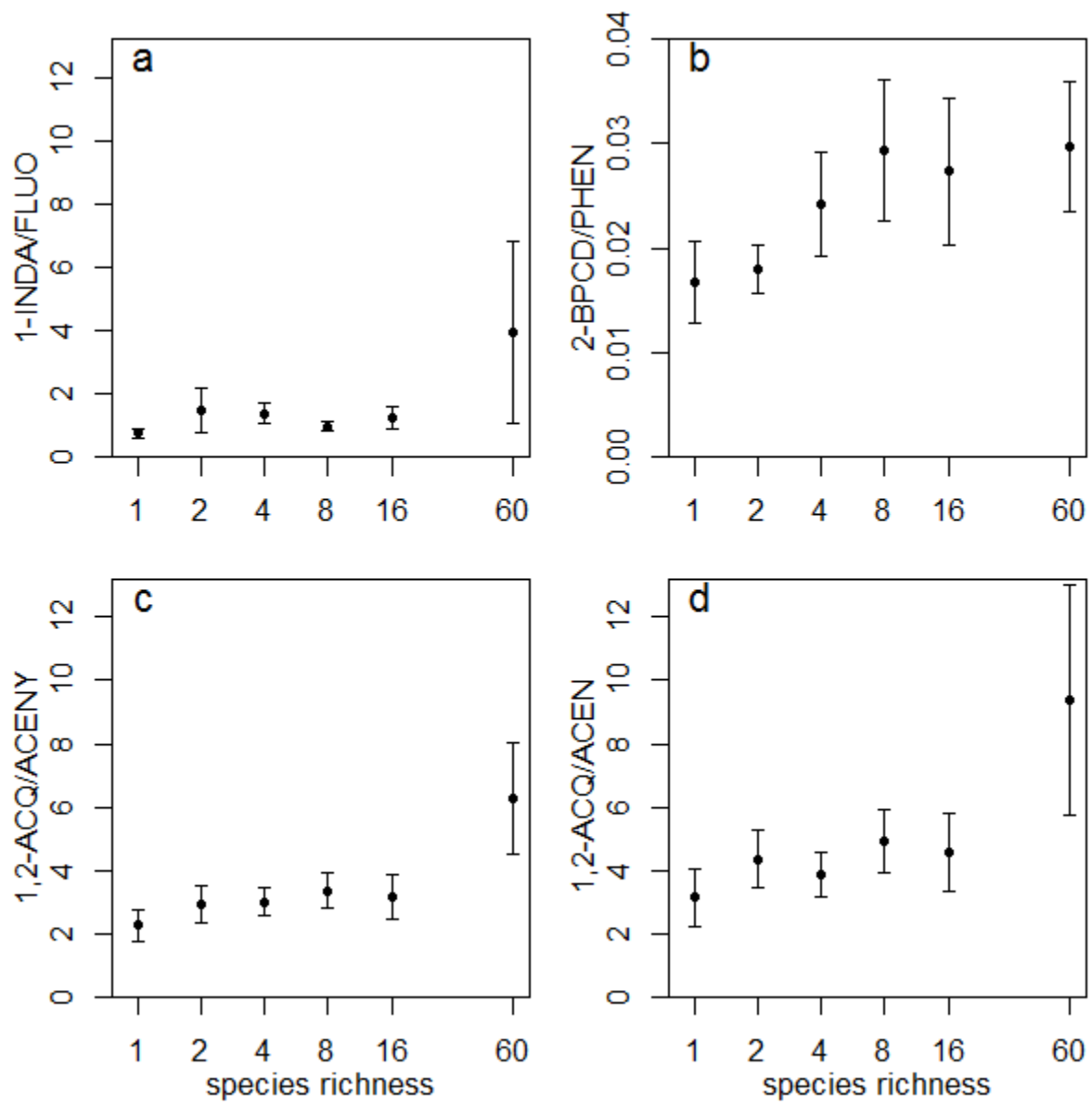
Figure 2



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Figure 3

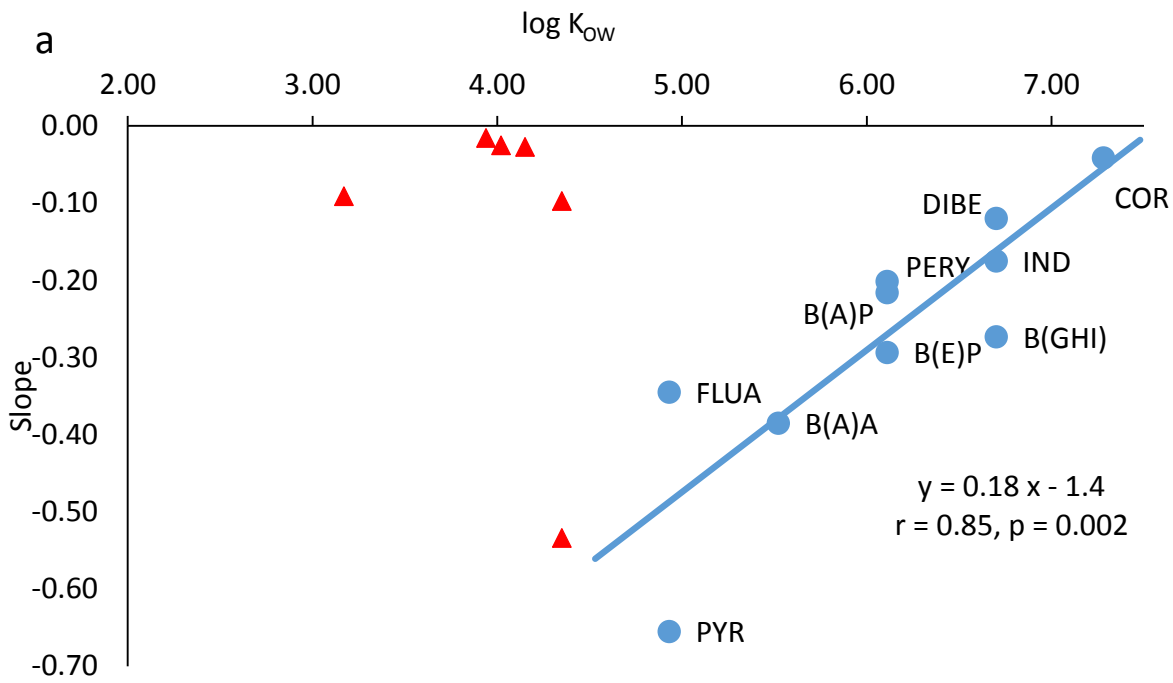


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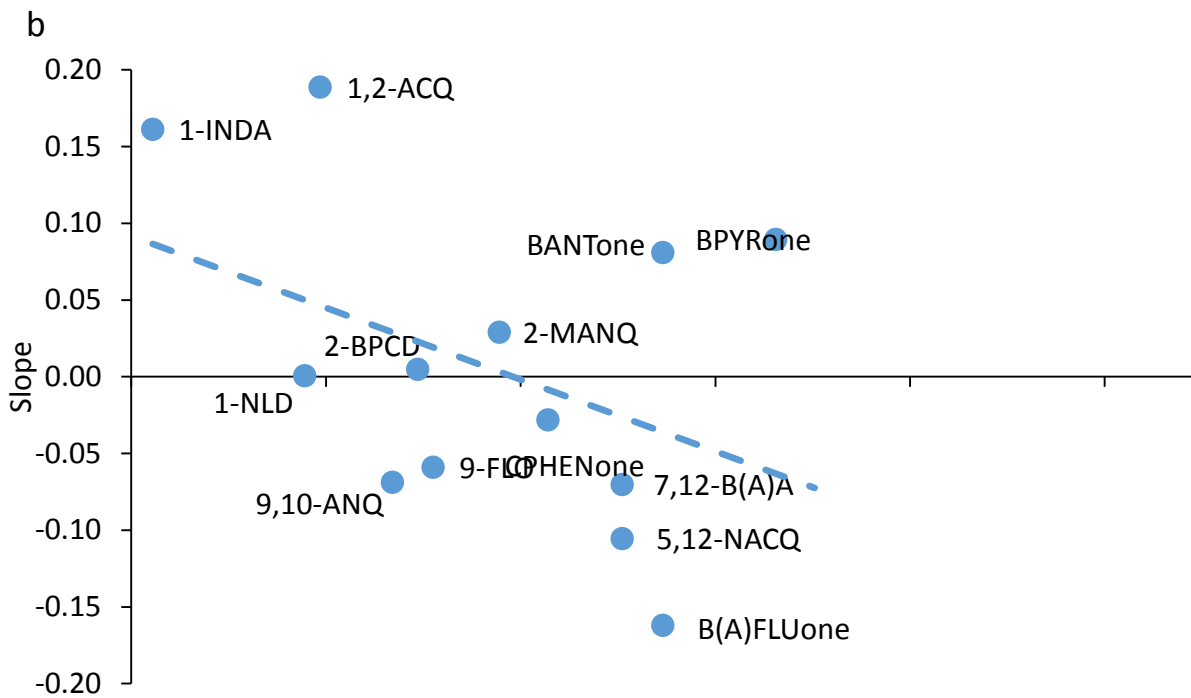
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Figure 4

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