Supporting Information

In Vitro Prediction of Polycyclic Aromatic Hydrocarbon Bioavailability of Incidentally Ingested Soil in Juvenile Swine

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Supporting Information

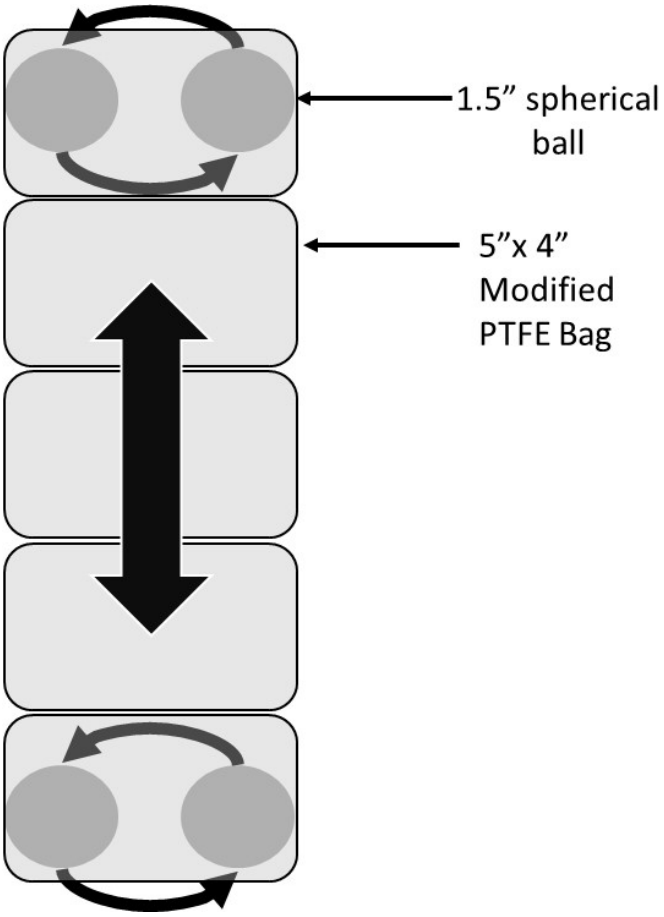

Figure S1. Diagram of the low energy FOREhST massaging method. In the method, 2-1.5” rotating spherical balls move up and down the length of 5 modified polytetrafluoroethylene (PTFE) bags (5”x4”).

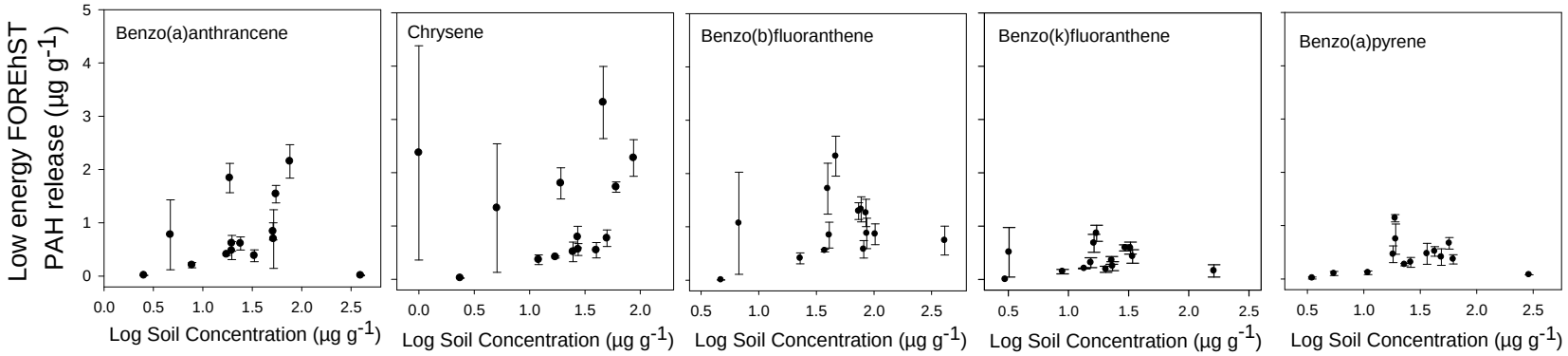

Figure S2. Comparison between low energy FOREhST PAH release and soil concentration of five PAHs in 14 soils historically contaminated with hydrocarbons. Data points represent the mean (n=3) for FOREhST PAH release and error bars represent the standard error of this mean.

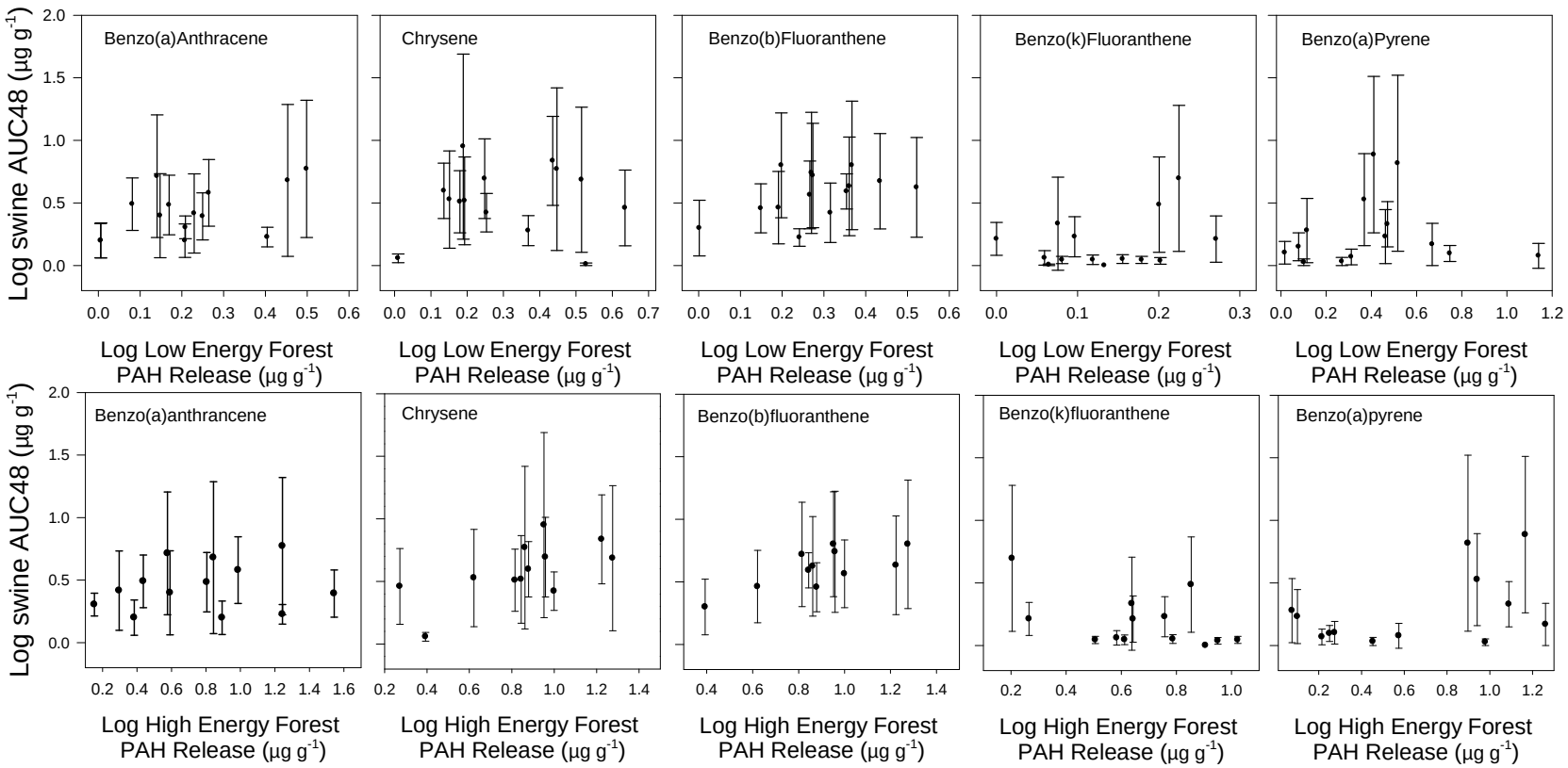

Figure S3. Comparison of Area Under the Curve (AUC) against Low energy FORE(h)ST PAH release (top) and high energy FOREhST release (bot) of 5 PAHs in 14 soils historically contaminated with hydrocarbons. Data points represent the mean (n = 6) of mammalian exposure (AUC48) measured in µg PAH per gram of soil and (n=3) estimates of in vitro FORE(h)ST PAH release. Error bars represent the standard error of the mean.

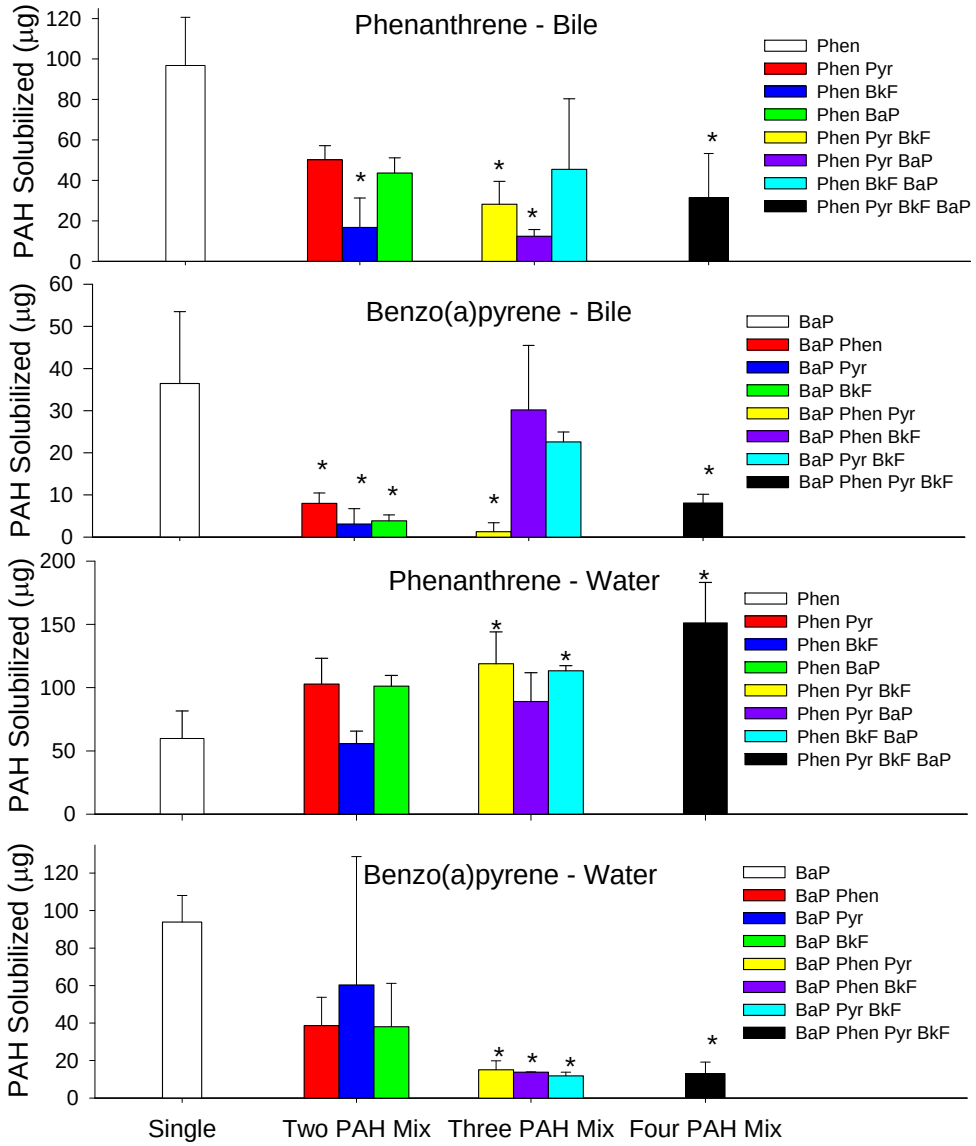

Figure S4. Comparison of phenanthrene and benzo(a)pyrene solubility in both bile (top 2 panels) and water (bottom 2 panels) from single compound to multiple compound mixtures. Significant difference is indicating by '*' at $p < 0.05$. Abbreviations are as follows: Phen is phenanthrene, Pyr is pyrene, BkF is benzo(k)fluoranthene, and BaP is benzo(a)pyrene.

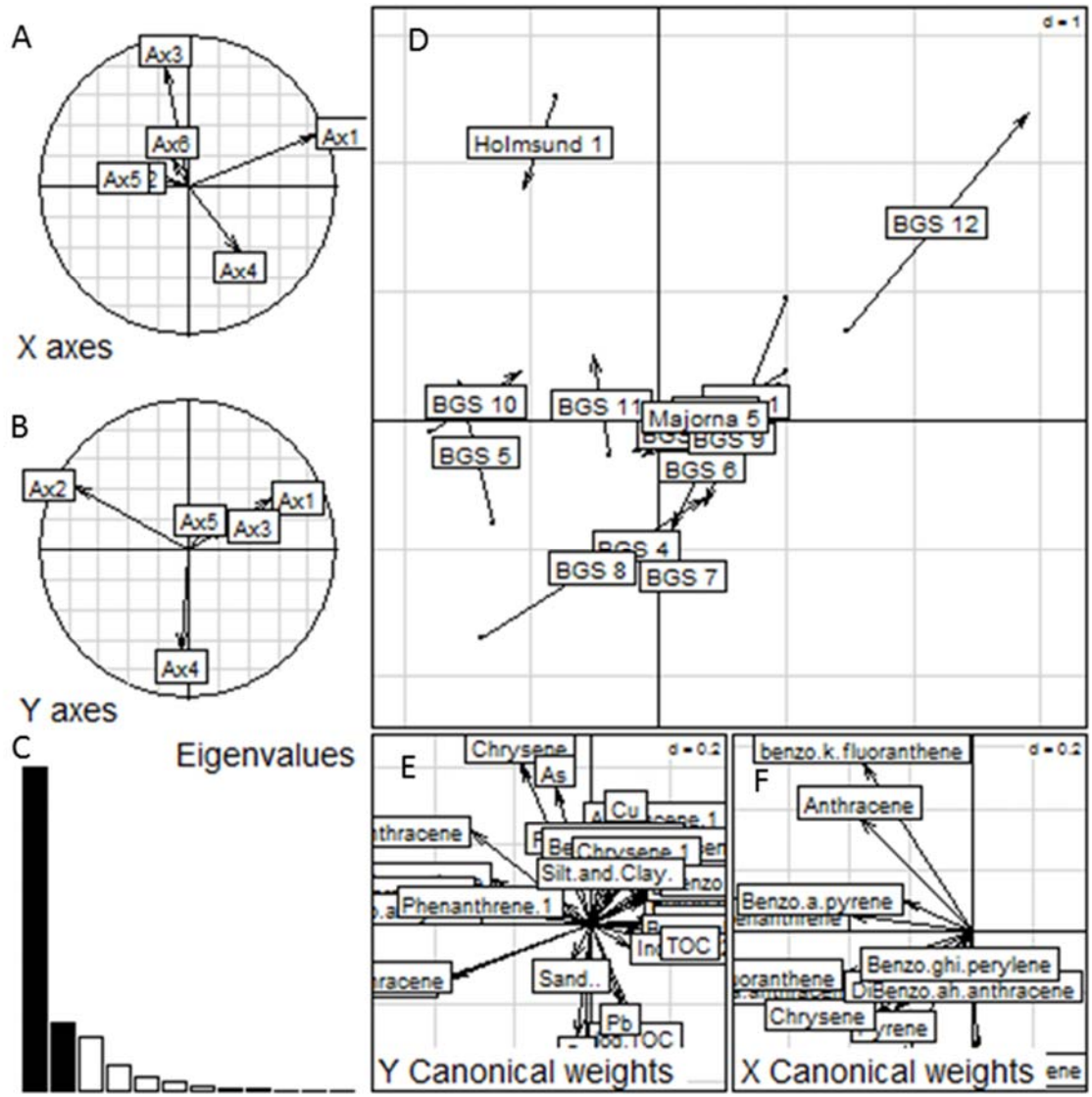

Figure S5. Output of co-inertia analysis. A) Components of the standardized principal component analysis of the PAH bioavailability data set projected on to the co-inertia axes. B) Components of the standardized principal component analysis on the predictor variable (PAH bioaccessibility and soil properties) data set projected on to the co-inertia axes. C) Historgram of the eigenvalues. D) Standardized co-inertia scores of the bioavailability (A) and predictor variable data sets (B) projected on to the co-inertia axes. E) Canonical weights of predictor variables F) canonical weights of PAH bioavailability

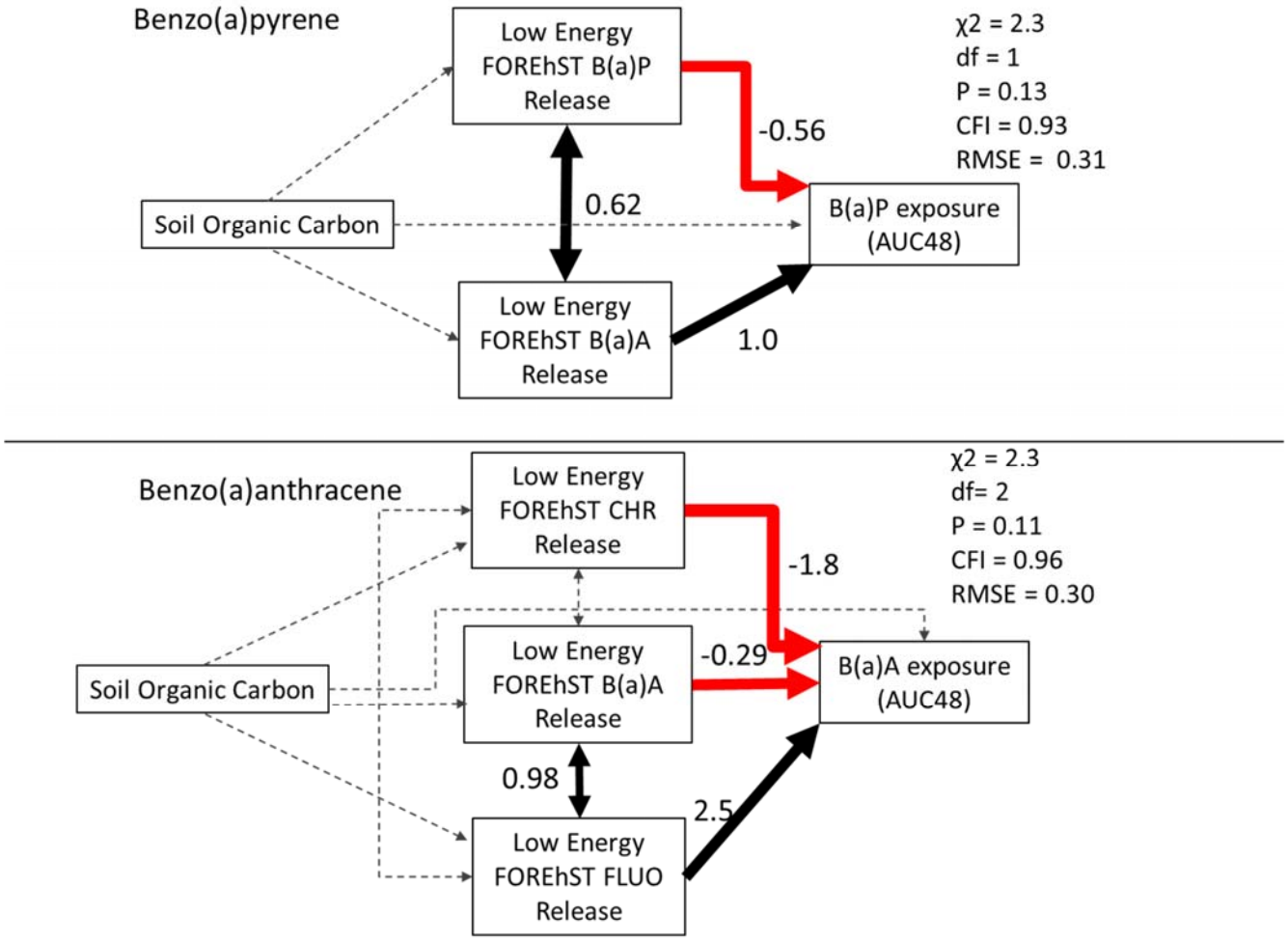

Figure S6. SEM diagram of the relationships between Soil Organic Carbon, FOREhST release of multiple PAHs, and PAH exposure. Single headed arrows indicate that a change in the variable at the tail causes a direct change to the variable at the head. Double headed arrows indicate non-directed causality. Dashed lines indicate a non-significant (P>0.05) path, whereas black arrows indicate a positive relationship and red arrows indicate a negative relationship. Arrow width corresponds to the strength of the relationship between variables with standardized coefficients provided for significant paths.

Table S1. Soil PAH release in the low energy FOREhST model across 14 soils

Soil	ANT[1]	FLU[1]	PYR[1]	B(a)A[1]	CHR[1]	B(b)F[1]	B(k)F[1]	B(a)P[1]	DIB[1]	B(g)P[1]	IND[1]
BGS1	0.0062 (0.0035)	0.10 (0.0040)	0.035 (0.0047)	0.015 (0.0023)	0.023 (0.0066)	0.0037 (0.00003)	NA	0.017 (0.030)	0.00076 (0.0013)	NA	0.00018 (0.00032)
BGS2	0.25 (0.060)	2.1 (0.62)	2.4 (0.68)	0.61 (0.21)	0.56 (0.19)	1.26 (0.45)	0.36 (0.13)	0.67 (0.19)	0.63 (0.16)	0.40 (0.16)	0.34 (0.13)
BGS3	0.17 (0.11)	1.9 (0.98)	1.4 (0.69)	0.48 (0.29)	0.51 (0.32)	0.88 (0.49)	0.25 (0.15)	0.47 (0.34)	0.52 (0.33)	0.32 (0.20)	0.25 (0.14)
BGS4	0.11 (0.026)	5.1 (1.2)	3.2 (0.85)	0.84 0.28)	0.77 (0.27)	0.86 (0.34)	0.43 (0.22)	0.37 (0.15)	0.53 (0.22)	0.38 (0.16)	0.33 (0.14)
BGS5	0.33 (0.044)	9.2 (2.5)	3.2 (2.6)	1.8 (0.48)	1.8 (0.51)	2.3 (0.65)	0.87 (0.26)	1.1 (0.12)	1.5 (0.33)	1.2 (0.29)	0.97 (0.30)
BGS6	0.11 (0.033)	3.0 (0.95)	2.3 (0.81)	0.62 (0.26)	0.79 (0.35)	0.85 (0.42)	0.31 (0.16)	0.46 (0.27)	0.50 (0.26)	0.43 (0.23)	0.38 (0.20)
BGS7	0.021 (0.012)	1.5 (0.57)	0.92 (0.37)	0.21 (0.083)	0.37 (0.16)	0.41 (0.18)	0.15 (0.071)	0.11 (0.060)	0.24 (0.11)	0.25 (0.11)	0.18 (0.081)
BGS8	0.045 (0.028)	2.3 (0.83)	1.1 (0.52)	0.38 (0.19)	0.55 (0.25)	0.58 (0.28)	0.19 (0.098)	0.31 (0.16)	0.31 (0.16)	0.22 (0.114)	0.19 (0.088)
BGS9	0.040 (0.0059)	1.4 (0.12)	0.98 (0.082)	0.41 (0.032)	0.42 (0.032)	0.55 (0.042)	0.20 (0.014)	0.27 (0.039)	0.31 (0.026)	0.23 (0.017)	0.20 (0.015)
BGS10	0.364 (0.096	13 (2.3)	0.00099 (0.0017)	2.2 (0.54)	2.3 (0.59)	1.33 (0.40)	0.59 (0.19)	0.52 (0.16)	0.58 (0.18)	0.52 (0.17)	0.48 (0.14)
BGS11	0.15 (0.081)	6.9 (0.20)	0.043 (0.019)	1.5 (0.29)	1.7 0.17)	1.3 (0.27)	0.59 (0.099)	0.41 (0.27)	0.60 (0.15)	0.52 (0.12)	0.53 (0.096)
BGS12	ND	ND	ND	0.012 (0.013)	2.4 (3.5)	0.74 (0.47)	0.16 (0.20)	0.078 (0.0013)	5.0 (1.3)	3.7 (1.2)	3.3 (1.0)
WP1	0.37 (0.65)	4.0 (6.9)	0.029 (0.050)	0.70 (0.95)	3.3 (1.2)	1.7 (0.83)	0.68 0.29)	0.75 (0.49)	0.24 (0.12)	0.14 (0.085)	0.15 (0.082)
GW2	0.083 (0.081)	3.0 (4.7)	1.8 (2.8)	0.78 (1.1)	1.3 (2.1)	1.1 (1.7)	0.51 (0.80)	0.10 (0.067)	0.74 (1.1)	0.37 (0.54)	0.38 (0.60)

[1]Abbreviations are as follows: ANT is anthracene, FLU is fluoranthene, PYR is pyrene, B(a)A is benzo(a)anthracene, CHR is chrysene, B(b)F is benzo(b)fluoranthene, B(k)F is benzo(k)fluoranthene, B(a)P is benzo(a)pyrene, DIB is dibenzo(a,h)anthracene, B(g)P is benzo(ghi)perylene, and IND is indeno(1,2,3-c,d)pyrene

Values in parentheses indicate the standard deviation from 3 measurements

NA – No estimate available

Table S2. Soil PAH release in the high energy FOREhST model across 13 soils

Soil	ANT	FLU	PYR	BAA	CHR	BBF	BKF	BAP	DIB	B(g)P	IND
BGS1	4.9 (3.0)	3.3 (2.2)	2.0 (2.1)	1.4 (1.0)	1.5 (0.89)	1.9 (1.3)	0.84 (0.53)	0.88 (0.92)	1.3 (0.87)	1.3 (0.79)	1.0 (0.64)
BGS2	29 (6.4)	24 (5.9)	25 (6.6)	6.8 (1.7)	6.0 (1.5)	25 (6.0)	7.1 (1.7)	17 (4.2)	19 (4.5)	16 (3.7)	12 (2.6)
BGS3	25 (2.9)	15 (1.9)	24 (2.7)	5.4 (0.69)	5.5 (0.62)	18 (2.4)	4.7 (0.63)	11 (1.3)	13 (1.9)	11 (1.5)	8.7 (1.1)
BGS4	39 (34)	26 (23)	24 (21)	8.7 (7.5)	8.1 (7.0)	14 (12)	5.1 (4.4)	7.7 (6.7)	9.4 (6.4)	8.5 (7.4)	6.5 (5.5)
BGS5	34 (4.4)	23 (2.9)	12 (2.0)	6.0 (0.85)	6.3 (0.84)	7.9 (1.1)	3.4 (0.43)	2.8 (0.70)	5.1 (0.60)	4.9 (0.63)	3.9 (0.54)
BGS6	30 (3.6)	14 (1.2)	4.1 (5.5)	0.42 (0.66)	9.0 (0.99)	8.2 (0.83)	3.1 (0.28)	0.26 (0.064)	6.4 (0.65)	5.5 (0.54)	4.5 (0.39)
BGS7	22 (20)	12 (9.8)	0.98 (1.7)	1.7 (1.6)	6.6 (5.8)	5.9 (4.7)	2.8 (2.4)	0.19 (0.17)	4.1 (3.1)	4.2 (2.7)	3.4 (2.2)
BGS8	25 (14)	14 (6.9)	1.6 (1.4)	2.8 (3.3)	8.0 (4.1)	7.4 (37)	3.4 (1.7)	0.64 (0.80)	5.0 (2.0)	4.5 (1.6)	3.7 (1.3)
BGS9	10 (1.8)	6 (1.1)	3.7 (0.84)	2.9 (0.72)	3.2 (0.52)	5.1 (1.2)	2.2 (0.41)	1.8 (1.0)	3.4 (0.74)	3.6 (0.73)	2.8 (0.62)
BGS10	82 (8.5)	74 (9.0)	28 (21)	17 (2.5)	18 (2.1)	14 (1.9)	6.1 (0.66)	6.9 (2.1)	8.8 (1.3)	8.4 (1.1)	7.3 (0.78)
BGS11	55 (2.6)	57 (2.6)	33 (1.6)	17 (0.75)	16 (0.70)	19 (0.83)	7.9 (0.33)	14 (0.66)	13 (0.62)	12 (0.34)	10 (0.40)
BGS12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
WP1	149 (14)	96 (9.2)	113 (17)	34 (4.0)	31 (3.3)	23 (3.0)	9.5 (1.2)	8.5 (1.8)	5.9 (0.74)	5.6 (0.82)	3.1 (0.39)
GW2	2.3 (1.1)	1.4 (0.68)	2.6 (1.7)	0.98 (0.52)	0.87 (0.45)	1.2 (0.69)	0.60 (0.32)	0.78 (0.50)	1.3 (0.76)	1.1 (0.61)	0.28 (0.30)

[1]Abbreviations are as follows: ANT is anthracene, FLU is fluoranthene, PYR is pyrene, B(a)A is benzo(a)anthracene, CHR is chrysene, B(b)F is benzo(b)fluoranthene, B(k)F is benzo(k)fluoranthene, B(a)P is benzo(a)pyrene, DIB is dibenzo(a,h)anthracene, B(g)P is benzo(ghi)perylene, and IND is indeno(1,2,3-c,d)pyrene

Values in parentheses indicate the standard deviation from 3 measurements

NA – No estimate available

Table S3. PCA of PAH exposure (AUC48)

	PC1	PC2	PC3	PC4	PC5	PC6
Phenanthrene	-0.12	-0.17	-0.09	0.20	-0.79	-0.36
Anthracene	-0.29	0.07	0.55	0.091	0.065	0.15
Fluoranthene	-0.28	-0.12	-0.33	0.29	0.039	0.70
Pyrene	-0.42	-0.19	-0.20	-0.0056	-0.10	0.11
Benzo.a.anthracene	-0.41	0.053	-0.053	-0.031	-0.25	0.071
Chrysene	-0.41	0.0076	-0.18	-0.15	0.14	-0.26
Benzo.b.fluoranthene	-0.38	0.30	0.071	-0.037	0.13	-0.26
benzo.k.fluoranthene	-0.19	0.16	0.62	0.25	-0.12	0.12
Benzo.a.pyrene	-0.18	0.08	-0.22	0.60	0.43	-0.40
DiBenzo.ah.anthracene	0.035	0.62	-0.21	-0.0018	-0.17	0.21
Benzo.ghi.perylene	0.061	0.64	-0.15	-0.06	-0.13	-0.016
Indeno.123.cd.pyrene	-0.31	-0.05	-0.00071	-0.64	0.11	-0.032
Standard deviation	2.12	1.48	1.31	1.10	1.06	0.800
Proportion of Variance	0.376	0.183	0.144	0.101	0.093	0.053
Cumulative Proportion	0.376	0.559	0.702	0.803	0.897	0.949

Table S4. PCA of predictor variables, including PAH bioaccessibility and soil properties

	PC1	PC2	PC3	PC4	PC5
FOREhST Phenanthrene	-0.077	-0.26	-0.32	0.018	-0.066
FOREhST Anthracene	-0.053	-0.35	0.13	-0.0076	0.076
FOREhST Fluoranthene	-0.038	-0.30	0.26	0.11	-0.24
FOREhST Pyrene	-0.068	-0.047	-0.093	0.55	0.28
FOREhST Benzo(a)anthracene	-0.048	-0.32	0.20	0.10	-0.22
FOREhST Chrysene	0.098	-0.30	-0.034	-0.20	-0.16
FOREhST Benzo(b)fluoranthene	-0.014	-0.38	-0.064	0.066	0.091
FOREhST Benzo(k)fluoranthene	-0.046	-0.38	-0.038	0.070	-0.077
FOREhST Benzo(a)pyrene	-0.058	-0.33	-0.032	0.14	0.29
FOREhST DiBenzo(ah)anthracene	0.24	-0.037	-0.055	0.0047	0.098
FOREhST Benzo(ghi)perylene	0.24	-0.050	-0.050	0.018	0.080
FOREhST Indeno(123cd)pyrene	0.24	-0.047	-0.050	0.0032	0.051
Soil Phenanthrene	0.19	-0.11	0.29	-0.034	-0.17
Soil Anthracene	0.25	-0.013	-0.049	-0.092	0.044
Soil Fluoranthene	0.24	-0.073	0.042	-0.074	-0.047
Soil Pyrene	0.24	-0.081	0.094	-0.057	-0.13
Soil Benzo(a)anthracene	0.25	-0.014	-0.013	-0.065	-0.013
Soil Chrysene	0.25	-0.021	0.0072	-0.057	-0.041
Soil Benzo(b)fluoranthene	0.25	0.0012	-0.024	0.021	0.071
Soil Benzo(k)fluoranthene	0.25	-0.0086	-0.021	-0.0062	0.022
Soil Benzo(a)pyrene	0.25	0.0067	-0.031	0.0053	0.046
Soil DiBenzo(ah)anthracene	0.24	0.0054	-0.036	0.10	0.048
Soil Benzo(ghi)perylene	0.24	-0.0011	0.0089	0.11	0.0063
Soil Indeno(123cd)pyrene	0.24	-0.0045	-0.0056	0.11	0.017
Total organic carbon	0.23	0.085	-0.068	0.087	0.010
Log total organic carbon	0.19	0.085	-0.090	0.25	-0.081
Sand	0.028	0.048	0.54	0.14	0.14
Silt and Clay	-0.027	-0.050	-0.54	-0.13	-0.14
As	0.042	-0.24	-0.16	-0.036	0.46
Cr	-0.047	0.071	-0.11	0.37	-0.35
Cu	0.054	-0.099	-0.14	-0.0031	-0.45
Pb	0.051	0.068	-0.095	0.55	-0.14
Standard deviation	3.98	2.53	1.68	1.49	1.27
Proportion of Variance	0.494	0.201	0.088	0.070	0.0500
Cumulative Proportion	0.494	0.695	0.783	0.853	0.903

Table S5. Summary of Canonical Weights from Co-Inertia Modelling

Predictive Factor	X Canonical Weight	Y Canonical Weight	Strength[1]
FOREhST Chrysene	-0.181	0.407	0.446
FOREhST Fluoranthene	-0.396	-0.144	0.421
FOREhST Anthracene	-0.308	0.235	0.388
FOREhST Benzo(a)anthracene	-0.356	-0.136	0.381
Soil [As]	-0.092	0.348	0.360
FOREhST Pyrene	-0.0162	-0.349	0.349
FOREhST Benzo(k)fluoranthene	-0.299	0.0815	0.310
Soil [Cr]	-0.0380	-0.285	0.287
Soil [Anthracene]	0.165	0.232	0.285
FOREhST Benzo(a)pyrene	-0.281	0.0376	0.284
FOREhST Benzo(b)fluoranthene	-0.258	0.112	0.281
Soil log.TOC	0.114	-0.252	0.277
Soil [Cu]	0.0855	0.259	0.273
Soil [Pb]	0.0756	-0.202	0.216
Soil [Benzo(a)anthracene]	0.134	0.163	0.211
Soil [Benzo(a)pyrene]	0.176	0.109	0.207
TOC	0.180	-0.0476	0.186
FOREhST Dibenzo(a,h)anthracene	0.160	0.0902	0.184
Soil [Chrysene]	0.114	0.142	0.182
Soil [Fluoranthene]	0.0323	0.179	0.182
Soil [Benzo(k)fluoranthene]	0.140	0.0987	0.171
FOREhST Phenanthrene	-0.161	0.0556	0.170
FOREhST Indeno(123,cd)pyrene	0.146	0.0839	0.168
Soil [DiBenzo(a,h)anthracene]	0.158	0.0103	0.159
FOREhST Benzo(g,h,i)perylene	0.139	0.0647	0.153
Soil [Benzo(b)fluoranthene]	0.137	0.0589	0.149
Soil [Pyrene]	0.0216	0.132	0.134
Soil [Benzo(g,h,i)perylene]	0.130	-0.0143	0.130
Soil [Indeno(123,cd)pyrene]	0.103	-0.0622	0.120
Sand	-0.0509	-0.0913	0.105
Silt and Clay	0.0496	0.0891	0.102
Soil [Phenanthrene]	-0.0750	0.0541	0.0925

[1] Strength calculated as the square root of the sum of the squares for the X and Y canonical weights (Strength = $\sqrt{(X \text{ canonical weight}^2 + Y \text{ canonical weight}^2)}$).

Table S6. Summary of Collinear Data from Structure Equation Modelling

PAH	Model Parameters					Significant Relationship	P-value	Coefficient	Standardized Coefficient
	Chi-square	DF	P-Value	CFI	RMSE				
B(a)A	4.5	2	0.11	0.96	0.30	$B(a)A_{FOR}$ predicts AUC	0.01	-4.1	-1.8
						CHR_{FOR} predicts AUC	0.088	-0.45	-0.29
						FLU_{FOR} predicts AUC	0.001	1.1	2.5
						TOC predicts AUC	0.62	0.014	0.075
						TOC predicts $B(a)A_{FOR}$	0.40	-0.004	-0.048
						$B(a)A_{FOR}$ -FLU_{FOR} covariance	0.01	2.1	0.98
B(a)P	2.3	1	0.13	0.93	0.31	$B(a)P_{FOR}$ predicts AUC	0.03	-4.1	-0.56
						$B(a)A_{FOR}$ predicts AUC	0.001	3.5	1.0
						TOC predicts AUC	0.62	0.021	0.075
						TOC predicts $B(a)P_{FOR}$	0.74	-0.003	-0.070
						$B(a)P_{FOR}$ -$B(a)A_{FOR}$ covariance	0.05	0.112	0.62

Abbreviations are as follows: FLU is fluoranthene, B(a)A is benzo(a)anthracene, CHR is chrysene, B(a)P is benzo(a)pyrene, FOR represents the bioaccessible fraction of a PAH from low energy FOREhST model, and TOC is the soil organic carbon