SUPPLEMENTARY MATERIALS FOR:

Soil microbial community composition and tolerance to contaminants in an urban brownfield site

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Materials and Methods

Study site and sample collection.

At the 12 sites, surface samples (0.1524 m) were collected by hand using disposable scoops, and subsurface samples (1.524–6.096 m) were collected using a hardened steel-core barrel lined with an acetate sleeve, driven every 1.524 meters (5 ft) below ground by a direct-push drill rig. The samples were collected according to EPA Method 5035A using sample-dedicated Terra Core kits (En Novative), by pushing the “T” bar into an undisturbed portion of the soil from the acetate sleeve. Four Terra Core sample containers were filled at each sample site and placed into a resealable bag. All samples were immediately labeled and chilled to 4 °C by placing them on ice in an insulated cooler. Then, they were hand-shipped under chain-of-custody control to the laboratory for chemical analysis.

Sample processing: environmental parameters & eDNA metabarcoding library preparation

Two rounds of polymerase chain reaction (PCR) were used to amplify three targeted metabarcodes from soil samples for microorganisms from the Earth Microbiome Project: 16S rRNA, 18S rRNA, and fungal ITS (FITS). Primers for each of the three assays were ordered from Integrated DNA Technologies (Iowa) as originally published. Additionally, Illumina Nextera Transposase Adapters were included on the 5′ end of the synthesized primer pairs: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG (forward) and GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG (reverse). The 20 µL PCR mixes contained the following: 4 µL of 5X GoTaq Flexi Buffer (Promega), 0.4 µL of 10 mM dNTPs, 1.6 µL of 25 mM $MgCl_2$, 1 µL of 10 µM forward primer, 1 µL of 10 µM reverse primer, 8 µg of

Bovine Serum Albumin (BSA, 20 mg/ml, VWR, Pennsylvania), 0.15 µL of GoTaq G2 Flexi DNA Polymerase (Promega), 4 µL of DNA extract, and 5.85 µL of sterile water. Cycling protocols for each assay began with a 3minute denaturing period at 95°C and ended with a single 10-minute extension step at 72°C. The three step cycling involved denaturation at 95°C for 30 seconds, annealing for 45 seconds (temperatures and cycle numbers given in Table S2), and elongation at 72°C for 60 seconds. PCR products from the 16S rRNA, 18S rRNA, and FITS primers were pooled into one mix in equal volumes. They were cleaned with MagBind® TotalPure NGS (Omega BioTek Inc) magnetic beads at a ratio of 1 (beads):1 (DNA) and following the manufacturer's recommendations to remove fragments shorter than 350 bp.

During the second round of PCR, we added sample-specific dual indexes and used the PCR products described above as templates. Each pooled PCR amplicon mix was used as a template for a different sample; consequently, one library was generated for each eDNA sample. The second round PCR mix (30 µL) consisted of the following: 6 µL of 5X GoTaq Flexi Buffer (Promega), 0.6 µL of 10 mM dNTPs, 2.4 µL of 25 mM $MgCl_2$, 1.5 µL of 10 µM forward primer, 1.5 µL of 10 µM reverse primer, 0.15 µL of GoTaq G2 Flexi DNA Polymerase (Promega), 3 µL of the pooled PCR amplicon mix, and 14.85 µL of sterile water. The forward and reverse primers, which included the remaining Illumina adaptor sequence, were: AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC (forward) and CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG (reverse), and Nextera DNA indexes (i5 and i7) for dual indexing (Illumina, 2020). PCR products were cleaned with Mag Bind® TotalPure NGS (Omega Bio Tek Inc) magnetic beads at a ratio of 0.8 (beads):1 (DNA), following the manufacturer's recommendations to remove fragments shorter than 300 bp.

Identifying collinear environmental parameters

Of the hydrocarbons and heavy metals assessed and quantified (Table S2), only a fraction were considered for statistical analyses (Table S3). Those that did not display sufficient variation between samples (e.g. all samples had the same concentration) were excluded. Furthermore, hydrocarbons and heavy metals that were highly correlated with each other were also not included. For this, we constructed a correlation matrix of environmental predictors in R and identified those that were strongly correlated with one another ($r > 0.5$) and statistically significant ($\alpha = 0.01$) (Table S3). These were excluded from statistical analyses, leaving arsenic, cobalt, chromium, lead, and benzo(a)pyrene to be included in our statistical models (Table S3).

Samples from the surface (0.1524 m) were classified as “contaminated” if their values exceeded regional environmental screening levels and guidelines set by the respective overseeing agency for one or more of the following: heavy metals, TPHs, and/or PAHs (Table S2). Agencies included the Environmental Protection Agency [5], California Department of Toxic Substances Control (DTSC, 2019), and the San Francisco Regional Water Quality Control Boards (California San Francisco Bay RWQCB, 2019) for residential soils. Samples from the surface were classified as “uncontaminated” if that was not the case.

Results

Levels of contamination in soil of an urban brownfield site

Of the 17 heavy metals, 3 petroleum hydrocarbons, and 16 polycyclic aromatic hydrocarbons we quantified, only two metals, one petroleum hydrocarbon, and four aromatic hydrocarbons exceeded the regional screening levels set by the EPA, California DTSC, and San Francisco RWQCB for residential soils. All exceedances were found in surface samples. Specifically, the arsenic levels of six surface samples (range: 0–0.43 mg/kg) exceeded regional screening levels established by the EPA (0.68 mg/kg; Table S2) and DTSC (0.11 mg/kg; Table S2). The lead concentrations of two surface samples (1.6–140 mg/kg) surpassed the DTSC-established level of 80 mg/kg. TPH-d levels of four surface soil samples (320–640 mg/kg) exceeded the preferred ESL residential soil concentrations of 260 mg/kg (Table S2). For PAHs, two samples (750-4,000 µg/kg) had concentrations higher than the EPA RSL for rbenzo[a]pyrene (110 µg/kg), one sample (140,000 µg/kg) exceeded EPA RSLs for benzo[b]fluoranthene levels (1,100 µg/kg), and three samples (4,600-46,000 ug/kg) exceeded EPA-RSLs for benzo[k]fluoranthene (11,00 µg/kg; Table S2). Three soil samples (4,400-62,000 ug/kg) contained indeno (1,2,3-cd) pyrene concentrations exceeding EPA RSL guidelines (Table S2).