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A deployable film method to enable replicable sampling of low-abundance environmental microbiomes

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Urbanizing global populations spend over 90% of their time indoors where microbiome abundance and diversity are low. Chronic exposure to microbiomes with low abundance and diversity have demonstrated negative long-term impacts on human health. Sequencing-based analyses of environmental nucleic acids are critical to understanding the impact of the indoor microbiome on human health, however low DNA yields indoors, alongside sample collection and processing inconsistencies, currently challenge study replicability. This study presents a comparative assessment of a novel, passive, easily replicable sampling strategy using polydimethylsiloxane (PDMS) sheets alongside a representative swab-based collection protocol. Deployable, customizable PDMS films designed for whole-sample insertion into standardized extraction kits demonstrated 43% higher DNA yields per sample, and 76% higher yields per cm^2 of sampler over swab-based protocols. These results indicate that this accessible, scalable method enables sufficient DNA collection to comprehensively evaluate indoor microbiome exposures and potential human health impacts using smaller, more space efficient samplers, representing an attractive alternative to swab-based collection. In addition, this process reduces the manual steps required for microbiome sampling which could address inter-study variability, transform the current microbiome sampling paradigm, and ultimately benefit the replicability and accessibility of microbiome exposure studies.

Keywords Indoor microbiome, Passive sampling, PDMS, DNA yield, Extraction efficiency, High-throughput sequencing

Microbiomes and human health

Environmental microbiomes, diverse and ecologically complex microbial communities associated with inhabitable spaces, interact with and impact human-associated microbiota and human health outcomes[1,2]. Within these interactions, microbiomes have been found to influence many complex relationships related to long-term human health outcomes, including organ development, modulating organ function, and immune response[1,2]. Advancements in high-throughput metagenomic sequencing[3] have improved our ability to explore the impacts of urban environmental microbiomes, which have characteristically low diversity and richness of many taxonomic groups[4], with negative impacts to human health[5]: Mechanically ventilated indoor spaces, where urban residents spend over 90% of their time[6], present low microbiome diversity and abundance[4] as compared to more informal[7] and non-urban settlements[4]. Indoor environmental microbiome exposures have subsequently been correlated with detrimental human health outcomes, where low abundance and diversity have been linked to increased instances of atopic skin conditions and allergy[8,9], asthma[8,10,11], obesity[11–13], cancer[14], depression[15,16] as well as the transmission of potential pathogens[17–19].

Although environmental microbiome diversity is only one variable by which microbial communities might be compared, existing research indicates it is a useful metric that appears to predict human health outcomes. For example, emerging research indicates relationships between environmental microbiome diversity and exposure with human health outcomes are reversible: while common building maintenance protocols such as HVAC

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use and surface cleaning contribute to reducing microbial diversity while increasing antimicrobial resistance in indoor microbiomes[20], environmental biodiversity interventions (such as vegetation[21]) can diversify indoor microbiomes with measurable benefits to human health[22]. Further study is required within this rapidly developing field to determine the mechanisms by which environmental microbiome diversity (as well as other metrics), human exposure, and ultimately interactions with human microbiomes might influence long-term health outcomes.

Metagenomics and sampling protocols

Indoor spaces in which urban residents typically spend 90% of their time[6] are a critical environment within which to understand microbiome exposure. These spaces are also challenging to study as they have characteristically low biological material[23], making it difficult to meet the DNA yield requirements for reproducible metagenomic analyses[23,24]. This has resulted in challenges of replicability for studies characterizing the microbiomes of urban indoor environments as different sampling and analysis protocols influence the quantification of the microbiome, and methodological differences often overshadow biologically relevant variation[25]. In addition, poor analytical outcomes due to low DNA yields[26] can translate to an unduly high signal for any present contaminants[24].

As the field continues to develop, strategies for avoiding, identifying, and removing contamination from analyses have emerged through a variety of methodological approaches[27]. Negative controls throughout both sampling and sequencing processes are necessary to identify contamination through DNA quantification, while sequencing analysis of negative control DNA is required to control for potential contamination within experimental samples analytically[27]. Computational approaches have also been proposed to re-analyze existing datasets and correct for analytic artifacts[28], as have alternative extraction methods for future work (such as a liquid–liquid approach[29]). Although all such developments are important protocols to utilize in future work, they do not address the challenge of insufficient DNA input to metagenomic processes, which may require an alternative sample collection method to those commonly employed in indoor studies.

Sample collection methods that have been employed thus far by indoor microbiome studies span a large range of protocols. Airborne bioaerosols have commonly been sampled through the use of active air flow sampling on filters[30], however this sampling approach is typically only representative of short time periods. Another common strategy involves collecting material that has settled on surfaces[31] which tends to be more representative of chronic exposures while capturing aspects of both airborne and dermal exposure, the latter of which is another major avenue of microbial transmission to humans[32]. Studies that sample microbiomes that have settled on surfaces often utilize swabs to collect existing biological material from horizontal surfaces[33], however this method presents challenges as the deposition timeline of material collected by swabbing existing surfaces are often unclear, and DNA extraction efficiencies from commercially available swabs are commonly limited to 15–35%[33].

Harmonization of both sampling and analysis stages may help address challenges of reproducibility in sequencing-based experiments. Not only do swabs have reportedly low extraction efficiencies[33], but sampling processes utilizing swabs as a sampling tool are likely inefficient: although swabs are often used to collect and transfer deposited biomass from surfaces of interest for downstream analysis, this process likely leaves biological material behind. Additionally, variability in individual techniques may result in differences in swabbing surface area and time, which could alter outcomes and further impact inter-study comparability. Although alternative collection materials to swabs have been explored, such as wipes[34,35], such a substitution requires a specialized extraction protocol[35], which does not allow for direct comparisons across studies[24], and does not fundamentally address sample timeline challenges. A variety of deployable surfaces, such as petri dishes[31] and common building materials[36], have been utilized within environmental microbiome studies to provide a controlled deposition timeline, however these methods continue to rely on swabbing the deployed material which fails to address the challenge of low swab extraction efficiencies.

A novel sampling approach

Passive deployable material that allows biological material to accumulate and be transferred in its entirety into an extraction pipeline may represent an alternative approach for assessing the indoor microbiome. Here we present a promising alternative to swab-based microbiome sampling: passive, deployable polydimethylsiloxane (PDMS) films that can be inserted in their entirety (along with any material deposited on its surface) into commercially available DNA extraction kits. PDMS is a biocompatible, chemically inert material[37] that has been used increasingly for work in direct contact with DNA such as microfluidic devices [38] for a variety of purposes, including solid phase extraction with direct amplification[39]. PDMS is an excellent material candidate for the proposed sampling technology because it is not only durable and flexible even when very thin[40] (allowing for insertion into extraction tubes), but it is inexpensive, chemically inert[37] and has been used in direct contact with nucleic acids without interference[39,41].

Passive, deployable PDMS samplers where the whole surface is inserted into commercially available extraction kits may address two challenges discussed thus far by (1) providing a clearly defined sampling timeline of surface deposition samples, and (2) increasing DNA yields as all captured material is utilized as input to processing and analytic protocols. Although promising, such an approach must be optimized in the field to allow researchers to collect DNA in quantities targeted for sequencing when collecting samples over extended periods: > 500 ng if standard sequencing approaches are utilized[23], as little as 1 ng if "ultra low" sequencing approaches are utilized[23,42]. PDMS has already been utilized in a similar manner to passively collect environmental chemicals[43] and respiratory viruses, including SARS-CoV-2[41], however DNA collection and extraction efficiencies for downstream sequencing have not yet been explored.

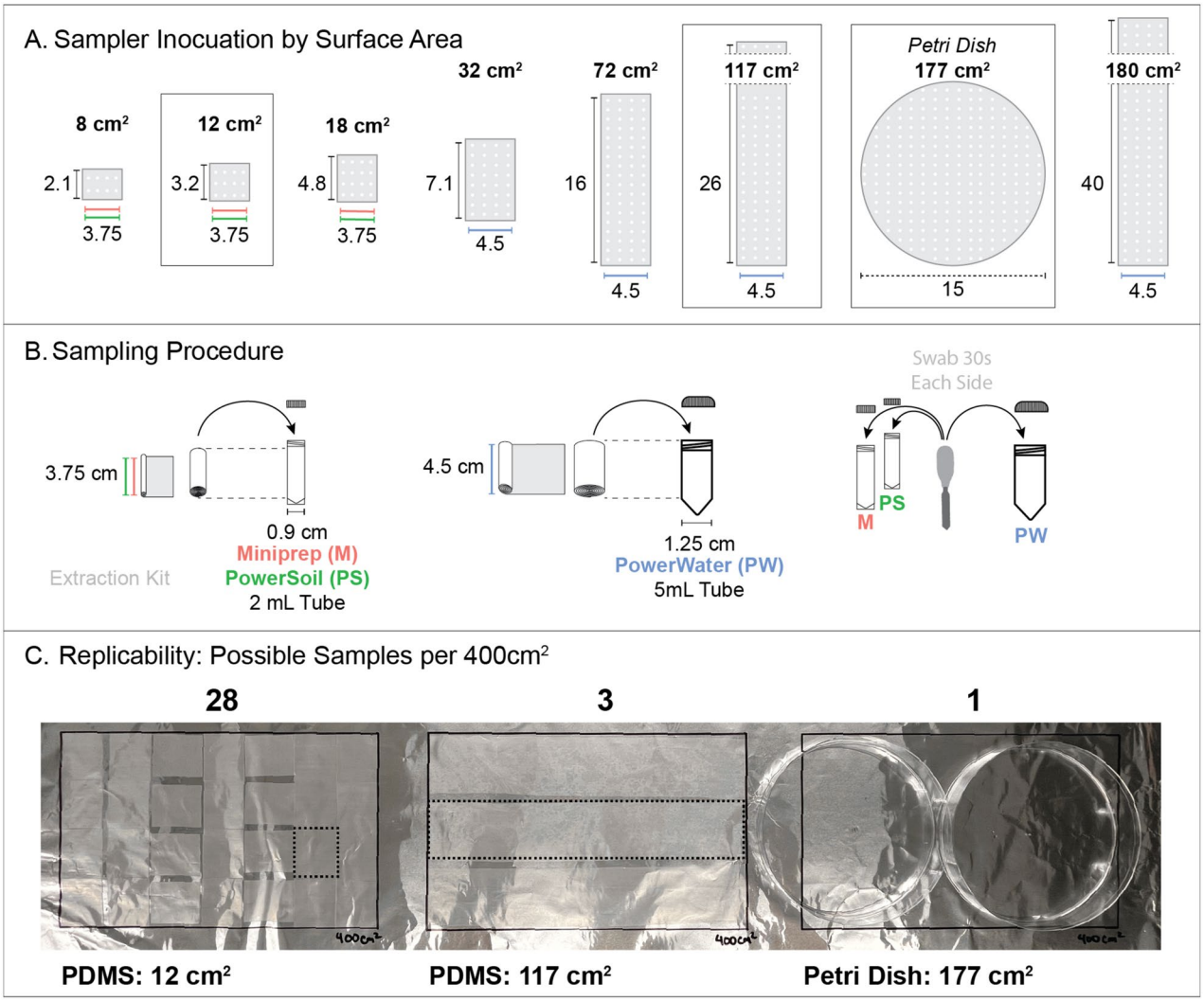

Fig. 1. (**A**) Illustrating the relative sizes of the eight samplers. White dots represent 1 µL of the diluted microbial community master mix. Samplers are drawn according to scale. Rectangular diagrams represent PDMS samplers in a variety of sizes inserted in their entirety into extraction kit collection tubes. The circular diagram represents a petri dish where collection required swabbing and inserting the swab head into extraction kit collection tubes. (**B**) Comparing PDMS with swab inputs and relationships to extraction bead-beating tube size constraints. (**C**) Comparing the field replicability of three samplers from section "A" (boxed) illustrating how the size and shape of each sampler impacts the number of possible sample replicates within a hypothetical deployment area of 400cm2.

Results & discussion

We present a preliminary benchmark study for an alternative to swab-based microbiome sampling that transforms DNA yield to facilitate sample replicability: deployable polydimethylsiloxane (PDMS) films, easily customized by the user (see Methods). The presented experiments compare DNA extraction yield, efficiency, yield per cm^2, and sequencing outcomes using a microbial community standard (Zymo Cat# D6300) between PDMS films in a range of sizes with a representative swabbed petri dish method as a preliminary investigation into the potential benefits of the described novel sampling technology. A deposition model was used to replicate microbiome biological material settling onto a surface with a standardized mixture: 1 µl of a diluted microbiome community master mix was pipetted onto each cm^2 of each sampling surface (Fig. 1A) and allowed to dry while covered. Three commercially available extraction kits were compared, referred to as "Miniprep" (MP), "PowerSoil" (PS), and "PowerWater" (PW). PDMS films were fabricated in the lab to optimally fill each extraction kit based on the height of the bead-beating tubes (Fig. 1B). A variety of lengths of the PDMS samplers were included to capture a range of sampler surface areas under each condition, where larger surface area resulted in both greater volume of the deposited standardized microbiome community, as well as a greater surface area of PDMS packed within the bead-beating tube (see Figures S 1–4). Swab samples were collected from commercially available 15 cm diameter petri dishes as a representation of a common swab-based sampling approach with which to compare the range of PDMS sampler protocols. Each Sampling/Extraction method was replicated three times.

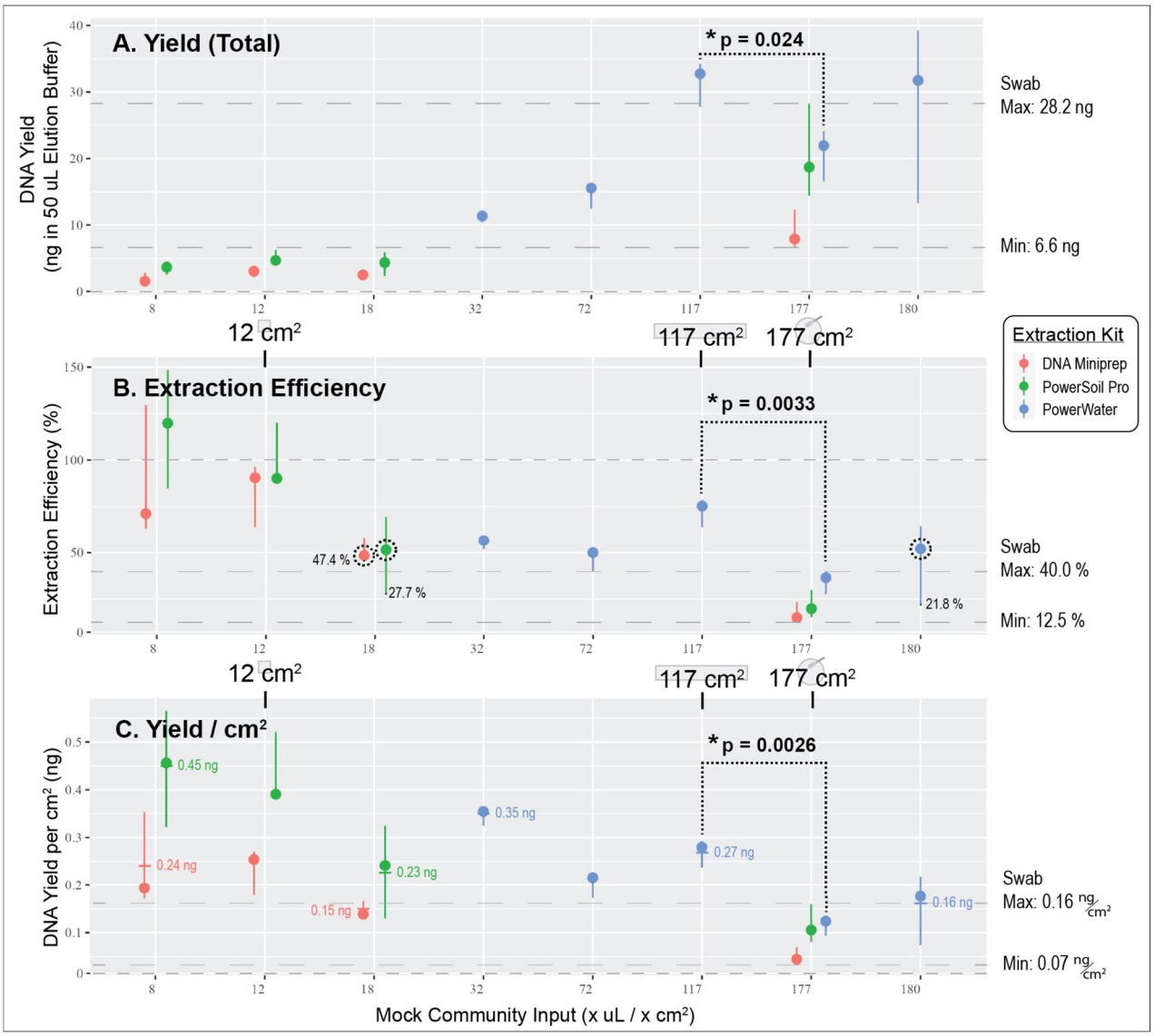

Fig. 2. (**A**) Line range plots of total DNA yield. (**B**) Line range plots of calculated extraction efficiencies in reference to positive controls (see Methods). Median values of the largest PDMS samplers included within each extraction kit are circled. The lowest quantified extraction efficiencies within each group are denoted. (**C**) Line range plots of DNA yield per cm2 of sampler area. Average DNA yield per cm2 is denoted for discussed samplers.

Sample replicability

As sampler size increased, sample replicability, as indicated by the number of samplers that can be deployed per area, decreased as a function of sampler surface area (Fig. 1C). Although field-deployable replicability was reduced for larger samplers, larger sampler sizes also resulted in greater DNA yields per sample (Fig. 2A). This relationship between sampler size, replicability per unit field area, and DNA yield per sampler indicates a clear cost–benefit analysis that must be considered where the available space within an identified field-deployment location, required sample number, and expected DNA yield must be weighed to optimize sequencing and analytic outcomes.

Extraction results: DNA yield

The largest average DNA yield corresponded with the 117 cm^2 PDMS/PowerWater protocol (31.6 ng, Fig. 2), which was 43% higher than the Swab/PowerWater mean yield (20.4 ng, Fig. 2) despite being 60 cm^2 smaller and receiving 60 µL less biological material than the swabbed petri dish (177 cm^2). The largest yield overall was collected from one of the180 cm^2 PDMS/PowerWater sampler/extraction protocols, however this group resulted in a lower average yield (28.8 ng) and was accompanied by the largest range in yield (+/− 13.0 ng). The smallest films sized for the Miniprep and PowerSoil kits all resulted in DNA yields 6.25 ng or below (Fig. 2), which are only suitable for "ultra-low" sequencing approaches that require as little as 1 ng of DNA[23,42], however the largest

average yield for this group was measured in the 12cm^2/PowerSoil protocol (5.2 ng). The presented yield results in combination with the replicability results (above) indicate that the 117 cm^2 PDMS/PowerWater group represents a promising sampling and extraction protocol that maximizes both DNA yields suitable for sequencing and sample replicability in the field.

Extraction results: extraction efficiency

Extraction efficiencies were calculated and analyzed in comparison to positive control yields within each extraction kit (see Methods) in an attempt to control for differences between extraction kits and explore the reported discrepancies in DNA yields between samplers despite differences in biological input. All PDMS films returned higher average extraction efficiencies than the swab samples extracted with the same kit ($p < 0.05$) with the exception of the 180 cm^2 PDMS/PowerWater method which was not statistically significantly higher ($p = 0.47$) (Fig. 2B). This result indicates higher extraction efficiencies of DNA from PDMS samplers (irrespective of extraction kit) likely contributed to the 43% greater DNA yield captured by the 117 cm^2 PDMS sampler than the corresponding swab samples, despite a smaller surface area and volume of biological material.

The extraction efficiency results within the PDMS samplers also provides preliminary evidence of an interaction between PDMS sampler size and extraction efficiency outcomes. Four of the smallest PDMS samplers (8–12 cm^2) extracted with the Miniprep and PowerSoil kits, returned extraction efficiencies over 100% in comparison to the positive control yields (see Table S1). This indicates PDMS sampler sizes that are small relative to the size of extraction kit bead-beating tubes may increase extraction efficiency, however due to the proximity of these samples to the limit of detection of the DNA quantification method (and thus a greater possibility of quantification error, see Methods) this result requires further exploration. In considering the impact of PDMS sampler sizes that are large relative to the extraction kit bead-beating tubes, the largest PDMS films resulted in the lowest calculated extraction efficiencies within each extraction kit (see Fig. 2B, circled). In addition, it was observed that these largest sized PDMS films filled the extraction kit bead-beating tubes nearly completely (see Figure S3 and 4). These outcomes indicate that extraction efficiency likely declines as a function of sampler size: small sampler sizes may somehow enhance extraction processes, perhaps by physically enhancing the bead-beating process, while larger volumes of sampler material may somehow inhibit the same processes or impede collection of the supernatant following the bead-beating step. Further testing is required to confirm the replicability of such patterns, as well as identify potential mechanisms.

Extraction results: DNA yield per cm^2

DNA yield per cm^2 was analyzed to more directly compare yield outcomes by controlling for input of the mock community biological material, which must be accounted for when considering both total DNA yield results and extraction efficiency calculations. The results support the finding reported in the extraction efficiency section whereby sampler size appears to impact extraction outcomes resulting in greater yields per cm^2 for smaller sampler sizes, and lower yields per cm^2 for larger sizes. Within the PDMS samplers, the greatest average DNA yield per cm^2 was calculated for the smallest sizes (Miniprep: 0.24 ng/cm^2, PowerSoil: 0.45 ng/cm^2, PowerWater: 0.35 ng/cm^2), while the lowest average DNA yield per cm^2 was calculated for the largest sampler sizes (Miniprep: 0.15 ng/cm^2, PowerSoil: 0.23 ng/cm^2, PowerWater: 0.16 ng/cm^2) and intermediate PDMS sizes resulted in intermediate yields (Fig. 2C, Table S1).

The 117 cm^2 PDMS sampler mean DNA yield per cm^2 (0.27 ng) was 76% greater ($p < 0.05$) than the mean DNA yield per cm^2 PowerWater swab samples (0.12 ng). Although this was not the highest calculated yield per cm^2, this outcome supports previous findings that the 117 cm^2 PDMS sampler/PowerWater extraction protocol, with greater DNA yield per cm^2 than a traditional swab-based method, represents a promising protocol that could be utilized to maximize DNA yield per cm^2 of deployed sampler area for sequencing-based experiments.

Sequencing results: negative controls & non-reference species

Negative controls, including sterile samplers and kit-blanks, were included for each sampler size and extraction kit (see Methods). All negative controls, including sterile samplers, resulted in DNA concentrations that were below the detection threshold (see Methods). Only one negative kit-blank sample, extracted with the Miniprep kit, was successfully sequenced (due to low DNA concentrations), therefore comparisons between extraction kits were not possible. In addition, this sample resulted in significantly more non-reference species than any experimental sample, likely due to analytic artifacts stemming from insufficient DNA[26]. Within the Miniprep negative sample sequencing results, 54 species were identified. 18 of these species were also identified in experimental samples, 36 were unique to the negative sample (see Table S2).

Within the 13 sampler/extraction kit protocols, all non-reference contaminant species were at an acceptably low level where even low-abundance reference species would not be excluded analytically. Within the 39 experimental samples the aggregated relative abundances for all non-reference species fell below 1% for 37 samples, with the two highest aggregated abundances falling at 3.1% and 1.2% for single samples (see Fig. 3). Analyzed on a species-specific basis, these are acceptably low contaminant abundances: Published literature indicates any species represented by less than 20% of the most abundant contaminant (in this case, non-reference) species should not be considered a true finding[44]. The lowest expected abundance of all the reference species was *C. neoformans* (0.37%, see Fig. 4), meaning the threshold relative abundance to disqualify this expected value was 1.85%. The most abundant contaminant (non-reference) species identified in this study was *C. acnes*. The protocol with the greatest *C. acnes* abundance was the 8 cm^2 PDMS/Miniprep protocol with a maximum of 3%, but an average of 1.05% (see Fig. 3, Table S3), the highest of which falls above the exclusion threshold, but the average of which does not. This pattern was confirmed within the data where *C. neoformans* relative abundances were often lower than the expected 0.37% (see Table SIII). Given these results, all reference species would be

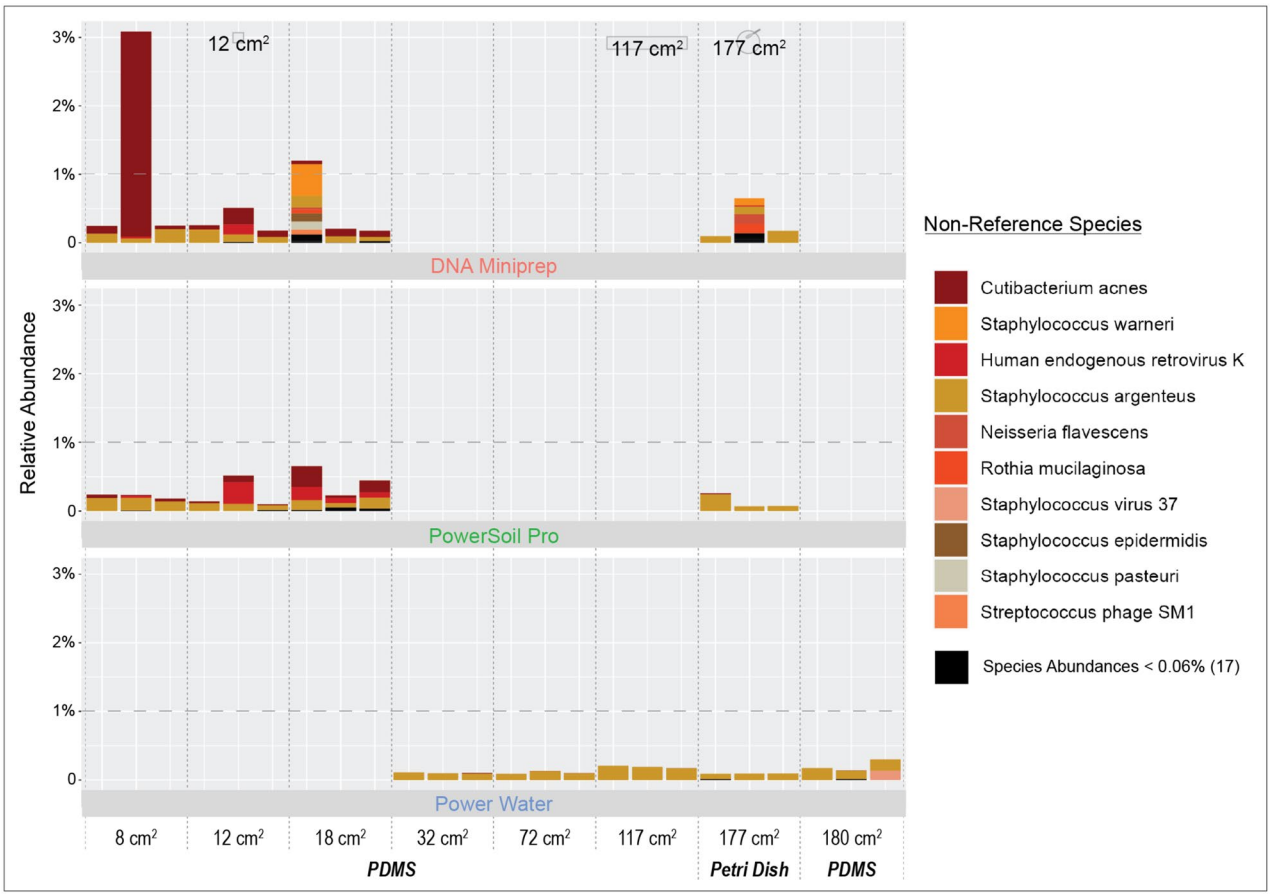

Fig. 3. Relative abundance (%) of the non-reference species identified by sampler/extraction kit protocols. A full list and average relative abundance by sampler can be found in Table SIV.

considered true findings for all protocols as long as exclusions were determined based on average outcomes for all identical sampler replicates.

Within the experimental samples, 27 non-reference species were identified, 9 of which were not identified within the Miniprep negative sequencing data (see Fig. 3, Table S4). In comparing the extraction kit outcomes, samplers extracted with the Miniprep kit resulted in the greatest number ($p < 0.05$) of non-reference species identified (21), followed by the PowerSoil samples (7), and the PowerWater samples (6). The Miniprep kit also resulted in the greatest average relative abundance ($p < 0.05$) of non-reference species (0.09%) in comparison to the PowerSoil (0.08%) and PowerWater (0.06%) kits, although all non-reference identification outcomes were within an acceptable range [44].

Sequencing results: reference species comparison & accuracy

Overall, the sequencing results did not differ significantly between the sampling strategies, however differences were identified between extraction kits. The PowerSoil extraction kit was the most accurate (5.1–7.8% dissimilarity from the reference), while the PowerWater kit was the least accurate (22.1–22.2%) (Fig. 4). In addition to community-level analyses, many of the species-level relative abundances differed between experimental groups. These results align with previous work that report differences in species abundance between extraction kits[28].

Concluding remarks

The results of this preliminary study indicate the combination of bespoke PDMS films and commercially available extraction kits represent an opportunity to establish a reliable environmental microbiome sampling protocol with greater DNA yields for downstream high-throughput sequencing analysis through greater extraction efficiencies and DNA yield per cm^2 of sampler, as well as improved field-replicability over a representative swabbed-petri dish protocol. Two promising protocols were identified: the 117 cm^2 PDMS/PowerWater protocol, and the 12 cm^2 PDMS/PowerSoil protocol. The 117 cm^2 PDMS/PowerWater protocol represents an opportunity to improve microbiome study outcomes under conditions where collecting sufficient DNA might be a challenge through increased DNA yield due to large sampler surface area and thus captured biological material, greater extraction efficiency and yield per cm^2 in comparison to the swab protocol, and through the use of the extraction kit with the least evidence of contamination. The 12 cm^2 PDMS/PowerSoil protocol might also represent an opportunity to improve microbiome study outcomes due to its use of the PowerSoil extraction kit, which represented the

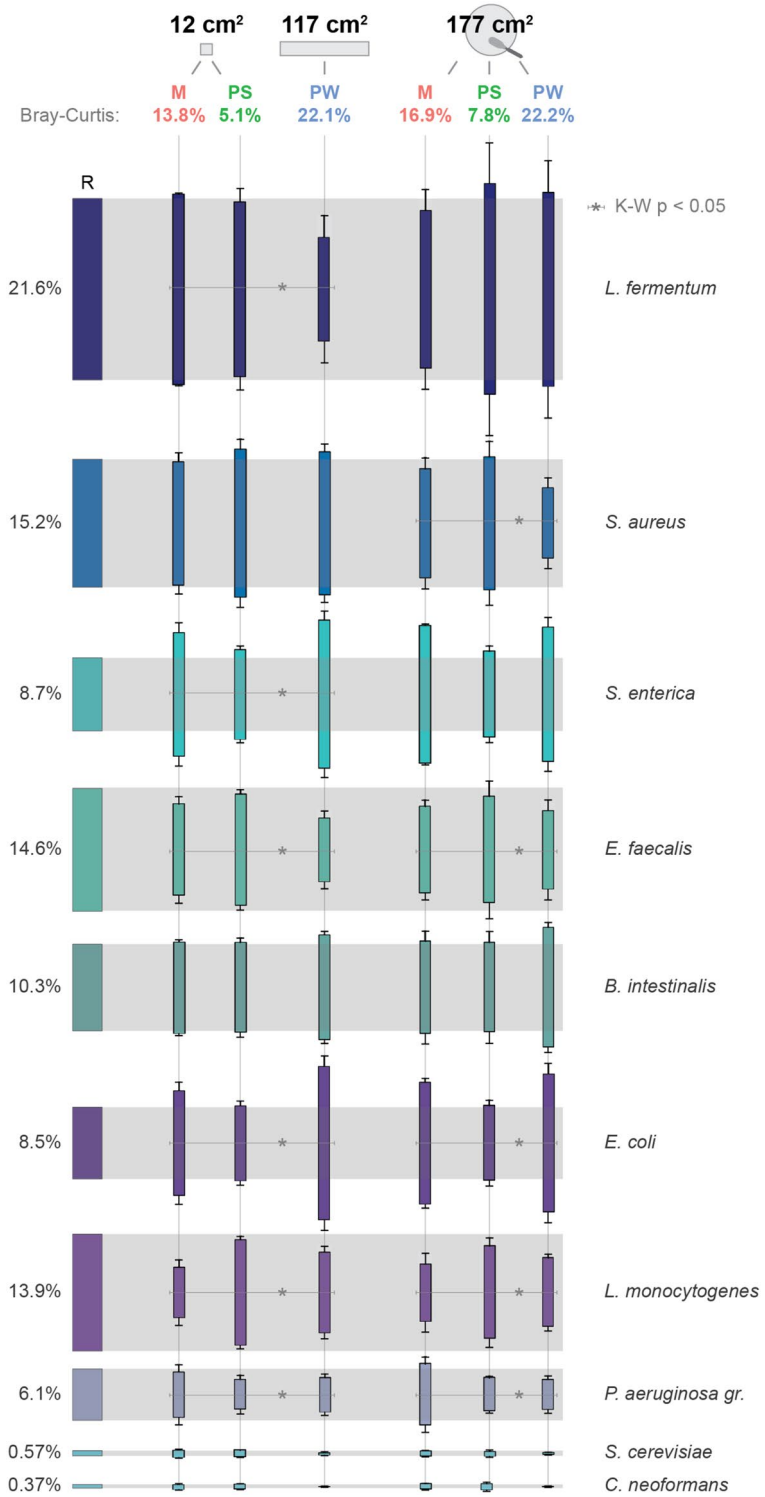

Fig. 4. A metagenomic analysis of the community structure for three representative samplers (Figure 1A&C). Outcomes were compared to the provided mock community reference numbers "R" (Bray–Curtis Dissimilarity), and species-level outcomes were compared within PDMS/Swab sampler groups (Kruskal–Wallis).

most accurate extraction kit based on the sequencing outcomes in comparison to the expected reference species abundance, however this protocol would likely require an experimental context where collecting sufficient DNA is less challenging (due to the lower surface area and captured biological material per sampler). Nevertheless, the 12 cm^2 PDMS/PowerSoil protocol would similarly provide improved field-replicability, and potentially reduce

sampling differences based on swab material or technique, as long as minimum DNA concentrations were achieved for the selected downstream analyses.

Although the presented results clearly indicate the potential value of adopting such approaches, it should be noted that the size and shape of the PDMS samplers could be altered to more uniquely fit the requirements of specific experimental needs without substantially altering outcomes, however more research is required to more fully understand how this novel protocol might be optimized, how outcomes may compare to alternative swab-based approaches that were not included in this study, and how such protocols may perform and compare to swab-based approaches under field conditions.

Future work & limitations

Flexibility in "Optimal" PDMS sampler size

Although a range of PDMS surface areas were included in this study under each extraction kit condition, further study is required to determine how replicable the presented results are, as well as how much each sampler size might be altered before significant differences are found in resulting extraction yields and efficiencies. A better understanding of the costs and benefits of marginal increases or decreases in length of the PDMS samplers around the two identified best performing protocols (e.g. 12 and 117 cm^2 sizes using the PowerSoil or PowerWater extraction kits) would help empower future researchers to fit uniquely dimensioned PDMS samplers to the needs of their selected application.

Direct comparisons to swab-based collection (laboratory & field conditions)

In building upon the finding that PDMS samplers can be optimally sized to maximize yield and efficiency outcomes within a single extraction kit, further study is required to more fully understand how the presented novel PDMS/extraction kit protocols might compare to a variety of swab-based approaches both under laboratory and field conditions. Future work should include a more systematic comparison of PDMS surface area outcomes to a range of petri dish sizes, swab materials, and swabbing techniques. Further study in the laboratory with standardized biological material would develop our understanding of how swabbed area, swab material, and swabbing technique may causally alter outcomes, and how such differences may compare to PDMS protocol outcomes. In addition, field testing will be required to determine how the presented PDMS protocols may impact study outcomes in comparison to common swabbing protocols, especially under conditions where researchers might expect different patterns of human occupancy, activity, available area to deploy samplers, and available microbial material, all of which might influence a researcher's ability to rigorously collect, quantify, and qualify the resulting DNA samples.

Extraction kits & sequencing outcomes

As discussed, extraction kit choice rather than sampler material choice (PDMS or Swab) had a statistically significant impact on resulting community composition metrics given standardized biological material, however these differences were consistent and within an acceptable range. This confirms previous findings that variations in extraction protocols should be considered when comparing microbiome studies[25]. Although the variability in sequencing results indicates room for improvement (Fig. 4), commercially available extraction protocols are rapidly evolving and will likely improve over time. In addition, consistent laboratory contamination confirms previous assertions that careful positive and negative controls are required, especially when studying environments with low microbial biomass and low sample DNA yields are expected[24]. If the presented methods are widely adopted, it is the authors' hope that the accuracy of extraction kits with large sample collection (bead-beating) tubes will improve to meet demand.

Implications

The process of deploying PDMS films to provide insight into indoor and urban microbiome exposures represents an opportunity to improve current microbiome research practices through greater DNA yields per sample, increased replicability within confined areas, and may create an opportunity for a new approach to sample collection altogether. Within indoor environments where biological abundance is low and collecting sufficient DNA can be challenging, the presented results indicate that the 117cm^2 PDMS film protocol would improve DNA yields per sample by 43% in comparison to the included representative swab-sampling technique (Fig. 2A). If such improvements in DNA yield were found to be consistent under field conditions, this outcome alone could drastically improve analytic outcomes[24,26]. In addition, greater DNA extraction efficiencies and yields per cm^2 in combination with the rectangular shape of the PDMS allow for smaller samplers to be tiled more closely together than petri dishes, which may improve inhabitant experience within occupied spaces (where desk space, for example, may be limited, see Fig. 5), and improve sample replicate numbers within a confined deployment area (see Fig. 1C). And finally, due the physical characteristics of PDMS, which is flexible, elastomeric, and can be reversibly deformed, seal to itself or a range of other materials, and conform to smooth surfaces[40], custom PDMS samplers may present an opportunity to more effectively sample limited surface area touch-based locations such as light switches or doorknobs[27], where PDMS might be reversibly deployed over and collected from such uneven surfaces, and DNA could be extracted directly from the entire sampler.

In addition to benefits the presented protocols might provide to future research techniques, the combination of customizable, deployable PDMS films would reduce human involvement (such as swabbing time or technique). This could reduce inter-study variability and ultimately benefit the replicability of microbiome exposure studies by simplifying microbiome sampling techniques. By simplifying microbiome sampling techniques, the PDMS film protocols may also represent an avenue for greater citizen science involvement in the study of connections between individual and environmental microbiomes with health outcomes, which is a popular topic in non-peer reviewed media where at-home kits are already commercially available in related fields[45].

Fig. 5. An example of the 117cm2 PDMS deployable film in triplicate within an indoor office space.

The presented combination of bespoke PDMS films and standardized, commercially available extraction kits (with sufficiently large tube volumes) represents an opportunity for greater control over microbiome sample collection that could be adopted immediately by any lab with standard equipment and could transform the accessibility of replicable indoor microbiome studies.

Methods

Samplers

Rectangular PDMS films were fabricated in the lab from 0.01 cm thick ISP™ PDMS (SSP-M823-Platinum Cured Silicone) using a sterilized rotary paper trimmer. The PDMS films were fabricated in seven different sizes (8, 12, 18, 32, 72, 117, and 180 cm^2) in widths corresponding to the heights of the respective extraction kit (Fig. 1A & B), laid on and covered by aluminum foil, and autoclaved. These PDMS films were compared to plastic, sterile petri dishes (15 cm diameter, 177 cm^2 surface area). Three experimental replicates were included for each sampler type.

Sampler inoculation & collection

A solution of ZymoBIOMICS™ Microbial Community Standard was diluted with sterile DNA-free water to achieve a targeted yield of 1 ng DNA/µl calculated from an expected yield of 2 µg DNA per 75 µl of the Zymo Microbial Community Standard (Cat# D6300). 1 µL of the diluted Zymo microbial community standard master mix was applied by pipette to each square centimeter of each experimental sampler (three each of the seven PDMS sizes, and petri dishes). The diluted microbial standard was vortexed between each inoculation to prevent settling and ensure consistent distributions. Inoculated petri dishes and PDMS films were allowed to dry while covered. Petri dishes were swabbed with Isohelix™ SK-2 rayon swabs for 1 min each (30 s on each side of the swab) after being moistened with the primary extraction liquid from each extraction kit. PDMS films were rolled with sterile tools and inserted directly into extraction tubes. A positive and negative control was collected for each sample size in each extraction kit by either (positive) directly pipetting the same volume of the diluted mock community into the requisite extraction kit collection tube or (negative) extracting a sterile sampler (i.e. without inoculation with the microbial standard).

DNA extraction

Each sampler type was replicated three times in three commercially available extraction kits: the by ZymoBIOMICS™ DNA-Miniprep extraction kit (Cat# D4300), and two Qiagen™ extraction kits: DNeasy® PowerSoil-Pro® Pro (Cat# 47016), and DNeasy PowerWater (Cat# 14900-100-NF). The optional PowerWater 65 °C 10 min incubation step was utilized, however the optional centrifuge step was not due to the need for specialized equipment. All samples were eluted in 50 µl of the provided final solution. Kit-blanks, bead-beating tubes with nothing added, were included for each extraction kit.

DNA quantification

DNA yield was quantified using an Invitrogen™ Qubit® 3.0 Fluorometer (Cat# Q33216). The level of detection of the Qubit® 3.0 Fluorometer under the than 20 pg/µL (which equates to 1 ng DNA in 50uL). Results are reported as total weight of DNA (ng) extracted within the 50 µl of the final solution. Extraction efficiencies of each sampler were calculated using the average DNA yield for positive controls extracted with the same extraction kit as the "expected" value. DNA yield per cm^2 of each sampler was calculated by dividing the sampler DNA yield by the surface area of the corresponding sampler.

Library preparation & sequencing

Libraries were generated with the Illumina DNA (M) Prep kit (Cat# 20018705) using a ¼ scale reaction volume throughout the library preparation. Shotgun sequencing was performed on an Illumina 6000 Novaseq system using an S1 cluster cartridge, a S1 flow cell, and a 300-cycle kit (Cat# 20028317). Sequencing parameters included 150 paired end base-pairs with dual 10 base-pair index reads.

Bioinformatics analysis

The MetaSUB CAP pipeline[46] was used for bioinformatics analysis using default settings. Raw sequenced data (pair-end reads) was processed with AdapterRemoval (v2.2.2)[47] to remove low-quality and ambiguous base reads, followed by Bowtie2 (v2.2.3)[48] human genome alignment (hg38, including alternate contigs). Read pairs with at least one mate that mapped to the human genome were discarded, the remaining reads were processed using the MetaPhlan3 (v3.0.7)[49] pipeline. The expected relative abundances of the reference species[50] identified using MetaPhlan3 species relative abundances were normalized to 100% following the removal of non-reference species. These updated relative abundances were then compared to the reference data provided by the Community Standard Protocol[50].

Statistical analysis

Statistical analyses were computed in R 4.2.2[51] with the vegan[52] and tidyverse[53] packages. The statistical tests utilized during analysis were as follows: Shapiro tests for normality, Levene's tests for homogeneity, Two-Sample T test for significance when normality and homogeneity assumptions were met, and Kruskal–Wallis tests when they were not. Data-driven graphics were created using ggplot2[54].

Percent difference between two values (V_1 and V_2) was calculated using the following equation:

$$\%Difference = \frac{|V_1 - V_2|}{\frac{V_1+V_2}{2}} * 100$$

Data availability

The data that support the findings of this study are openly available in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) data repository at: https://www.ncbi.nlm.nih.gov/sra/PRJNA1081427 ; BioProject ID: PRJNA1081427.

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Author contributions

All authors contributed extensively to the work presented. A.H.D. and E.M.H. jointly supervised the work. P.M.L, E.Z.L, K.J.G.P. , A.H.D., and E.M.H. jointly conceived the presented sampling concept. P.M.L. and E.M.H. designed the experiment. P.M.L. conducted the experiment. P.M.L. analyzed the DNA yield data. C.B. and E.M.H. conducted the metagenomic analysis. P.M.L., under the supervision of A.H.D. and E.M.H., created the figures presented in both the main and supplemental documents. P.M.L. prepared the manuscript. All authors contributed to editing the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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