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Emission Factors of Microbial Volatile Organic Compounds from Environmental Bacteria and Fungi

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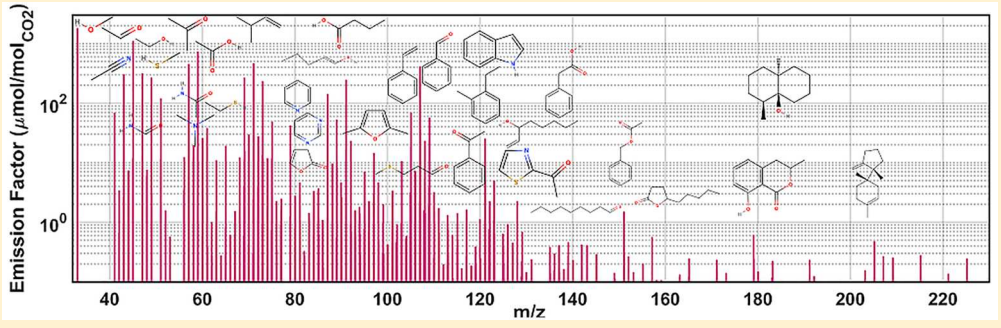
Supporting Information



ABSTRACT: Knowledge of the factors controlling the diverse chemical emissions of common environmental bacteria and fungi is crucial because they are important signal molecules for these microbes that also could influence humans. We show here not only a high diversity of mVOCs but that their abundance can differ greatly in different environmental contexts. Microbial volatiles exhibit dynamic changes across microbial growth phases, resulting in variance of composition and emission rate of species-specific and generic mVOCs. In vitro experiments documented emissions of a wide range of mVOCs (>400 different chemicals) at high time resolution from diverse microbial species grown under different controlled conditions on nutrient media, or residential structural materials (N = 54, $N_{control}$ = 23). Emissions of mVOCs varied not only between microbial taxa at a given condition but also as a function of life stage and substrate type. We quantify emission factors for total and specific mVOCs normalized for respiration rates to account for the microbial activity during their stationary phase. Our VOC measurements of different microbial taxa indicate that a variety of factors beyond temperature and water activity, such as substrate type, microbial symbiosis, growth phase, and lifecycle affect the magnitude and composition of mVOC emission.

INTRODUCTION

Microorganisms covering a broad range of metabolic and phylogenetic diversity are known to produce volatile gases affecting the chemical composition of air. The density of microbes in air can vary dramatically. Indoor air, for instance, contains between 10^2 and 10^9 microbes per dm^3 of air volume, and a similar number of microbes can be found coating every cm^2 of indoor surface.[1] Exposure to microbial VOCs in a residence can therefore also span a wide range of different chemicals although in general little is known about VOC emission activity of microbes in typical (nonsick) houses or their impact on human health.

Microbes need energy in order to grow and multiply, and they utilize sophisticated chemical machinery to transform available substrates into viable energy sources and to produce microbial biomass. Metabolic energy can also be shifted to secondary metabolism (e.g., toxin/antibiotic biosynthesis) in the presence of microbial competitors. In fact, up to a quarter of microbial genes encode enzymes for the production of various other molecules that are used in chemical signaling.[2,3] During microbial metabolism, nonvolatile, semivolatile, and volatile compounds can be released from microbial cells. Indoor environments offer a unique habitat where mVOCs are often recognized by human olfactory senses, and can trigger allergenic responses.[4,5] The accumulation of mVOC indoors requires abundant microbial activity which can be enhanced by favorable growth conditions and low air change rates. Indoor concentrations of any single mVOC reported to date have spanned nearly 6 orders of magnitude in concentrations,

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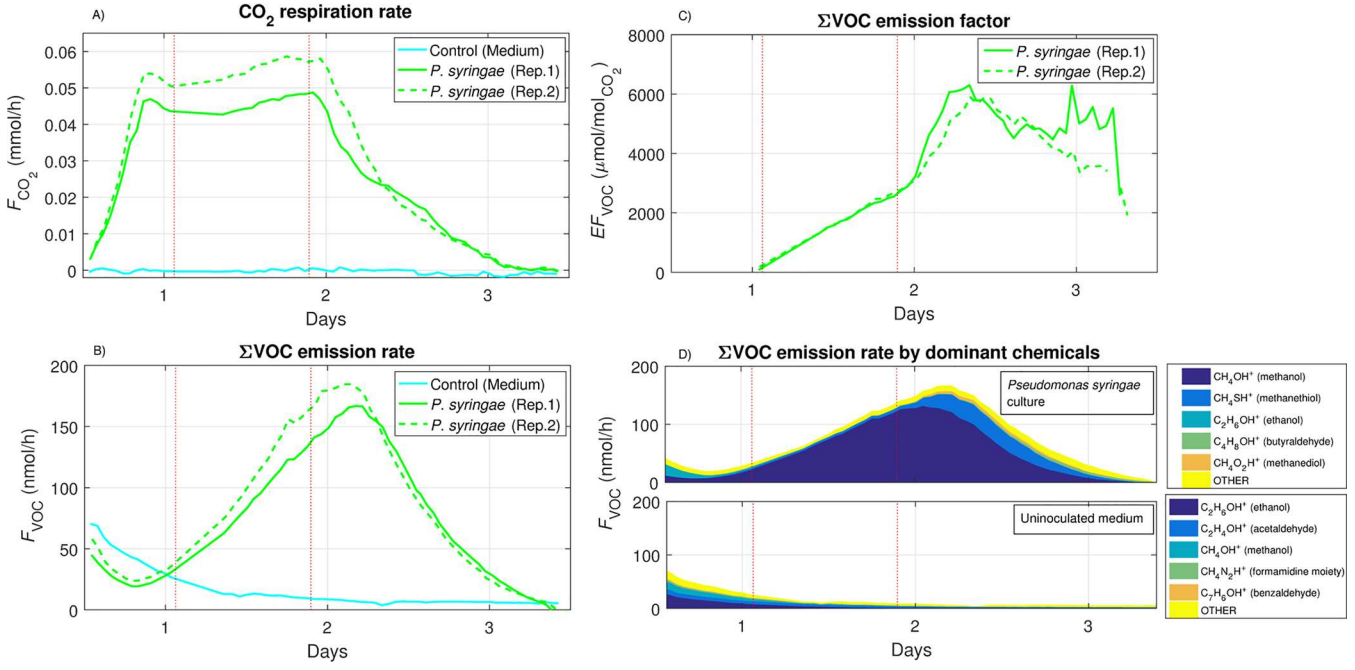

Figure 1. Growth phase-dependent respiration rate (a), ΣVOC emission rates (b), emission factor (c), and mVOC emission rates broken down by dominant compounds (d). The red dotted lines show the bounds of the stationary phase.

ranging from several ng m^{-3} to 1 mg m^{-3}.[6] Still, little is known about mVOCs related to indoor human exposure despite investigations of "sick buildings"[7] and indoor compost effluents.[8,9]

Humans are able to detect some mVOCs with low odor thresholds, such as the fungal mVOCs dimethyldisulfide, methylborneol, or bacterial sources of methanethiol and methional. The characteristic earthy scent referred to as petrichor in anticipation of rainfall is due to geosmin emanated mostly from sporulating soil actinobacteria, but can also be produced by other Gram-positive bacteria and secondary metabolism of fungi.[10] Some fungal mVOCs have recently been suggested as harmful, even at low concentrations, and therefore should be regarded as mycotoxins, contradicting a common assumption that mycotoxins are exclusively nonvolatile.[11] There is an evidence that 1-octen-3-ol, a prevalent fungal mVOC, can interfere with dopamine neurotransmission in humans which could lead to neurodegeneration.[12] Furthermore, specific mVOCs can highlight the presence of related nonvolatile mycotoxins on indoor surfaces, food, or hidden indoor spaces.[11]

A large portion of indoor microbial diversity is associated with humans, in part transferred from the complex human microbiome that covers about 1.8 m^2 of skin surface per person,[13,14] and comprises thousands of microbial species from 19 phyla.[15] The density of microbial cells on skin can rise dramatically upon transition from a planktonic to a biofilm state, a process mediated by quorum-sensing.[16] Indirect short-term human activities such as showering (providing conditions for microbial growth), leaving food out, or indoor composting[17] also can shape the microbiome of indoor spaces. In typical well-ventilated spaces, some of the emitted mVOCs are often present at such low concentrations they cannot be clearly detected without highly sensitive instruments.[6] However, by understanding emission rates of these compounds from microbial cultures, insights into the microbial activities and environmental conditions contributing to their production can be understood. Individual mVOC tracers used in some past studies[6] have shortcomings in that they are general and cannot be distinguished from other sources (e.g., plant vs microbe vs pollutants) demonstrating the need for measurement of more complete mVOC fingerprints to serve as specific tracers.[6]

The majority of indoor mVOC measurements have been conducted through time-integrated sampling, often referred to as offline sampling, which typically uses adsorbents to trap VOCs followed by analysis in a laboratory by gas chromatography. Over the last several decades measurements in indoor habitats have identified more than 200 mVOCs, 96 of which have been regarded as typical mVOCs, but only 15 of these have been evaluated in toxicological studies.[3,6] This approach has formed a useful base to recognize some abundant constituents of microbial odors, but better understanding of the factors affecting the abundance and time-resolved variability of mVOCs from microbes is still needed.

In this work, we use time-resolved measurements of the complete VOC high-resolution mass spectrum (1.00−500.00 amu) observable by proton transfer reaction ionization[18] during incubation of individual and interacting microbes. The aim of the study was to understand why and how mVOC are emitted from different microbial species isolated from indoor microbiomes. Addressing these fundamental questions is important for understanding processes behind emissions of microbial VOCs, for providing initial estimates of microbial source strengths, and developing knowledge useful for process-based modeling of mVOCs in the human environment. This is the first such comprehensive evaluation of mVOC emission strengths from a wide range of taxa of environmental microbes.

RESULTS AND DISCUSSION

Variability of Composition and Emission Rates of mVOCs over Time of Growth. We conducted measurements of a full mass spectrum of mVOC emissions from microbial cultures in concurrent replicate experiments. Figure

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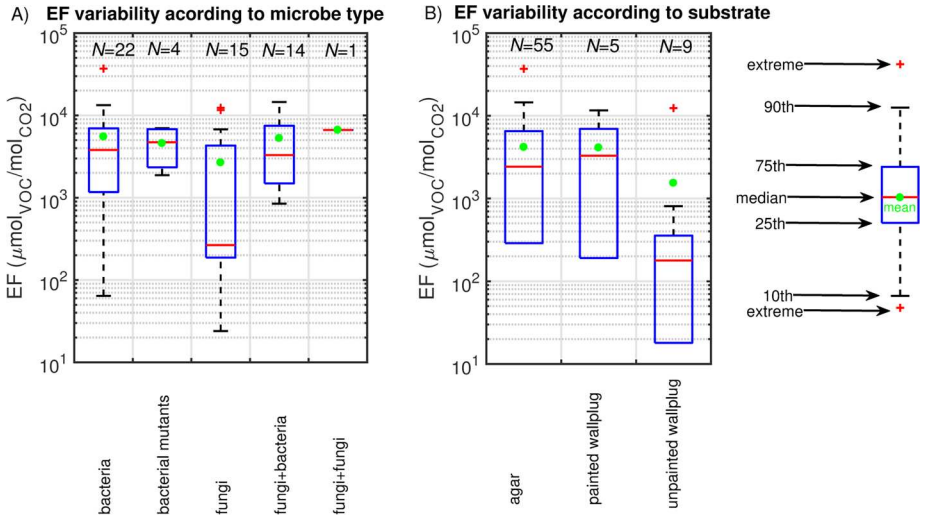

Figure 2. Variability in TVOC emission factors (derived by summing EFs for 469 ions) from stationary phases of 56 microbial strains according to community structure (a) and substrate type (b).

1 demonstrates an example of *Pseudomonas syringae* full-lifecycle emissions (i.e., from time of plate inoculation until maximal populations were achieved and through the decline phase). We found that mVOC emission rate is tightly coupled to CO_2 respiration rate and expected that the mVOC emission should also be dependent on the total microbe cell count. It is clear (Figure 1a, b) that this is indeed the case based on the differences in CO_2 respiration and mVOC emission rates between the replicates. The first 10 h of incubation are characterized by exponential increase in respiration, probably associated with rapid bacterial multiplication, followed by 2 days of reducing emissions and respiration. During stationary phase, microbial abundance is presumed to be relatively constant, but can differ between replicates. The mVOC emissions clearly undergo large changes in magnitude from the beginning to the end of the lifecycle of the plate culture and are rather independent of microbial abundance at various stages of microbial growth/activity. By normalizing the media-subtracted emission rate by the respiration rate, we defined the mVOC emission factor (EF), as in eq 1,

$$\mathrm{EF}_{\mathrm{mVOC}} = \frac{(C_{\mathrm{VOC}}(\mathrm{microbe}) - C_{\mathrm{VOC}}(\mathrm{medium}))*f}{F_{\mathrm{CO_2}}(\mathrm{microbe})} \quad (1)$$

where c_{VOC} denotes concentration in the chamber, f is the flushing rate of zero air, and $F_{\mathrm{CO_2}}$ is the respiration rate.

Although the mVOC emission rates differed in magnitude between replicates due to different numbers of cells, the EF accounts for microbial activity and shows consistent behavior of the two replicates in the stationary phase. Respiration rate provides an approximation of cell abundance during the logarithmic and stationary phase which correlates with mVOC emission, but mVOC emission is not clearly coupled to metabolic respiration rate after transition to the decline phase. This can be explained by increased oxidation during cell aging[19] and the increased turnover of the cells.

Chemical signaling via quorum sensing *N*-acyl homoserine lactones in *P. syringae* can result in changes in metabolite production including regulation of endogenously generated cytotoxicity and response to stress.[20] However, it is unknown how volatile metabolites are affected by quorum sensing mediated responses. The average EF during stationary phase represents approximate emission strength per cell abundance and is a useful quantity to estimate emission of mVOCs from similar microbes, if their surface density or respiration rate is known. In the case of *P. syringae* the average EF for total mVOC was on average 0.2% on a molar basis of the respired CO_2 in the stationary phase. As the emission in *P. syringae* is dominated by methanol, the mVOC percentage of respired carbon is similar. This is a particular case for methanol as the mVOC emissions in other microbes were not dominated by methanol but often by compounds with higher carbon numbers. For comparison, the EF averaged across all measured taxa was a factor of 2 higher on a molar basis (0.4%) and even higher (1.2%) per carbon basis. The higher average EF per carbon basis is due to emissions dominated by compounds containing a higher number of carbon atoms in a molecule. In the case of *P. syringae* (Figure 1), the density of bacteria was estimated at $0.5-1.2 \times 10^6$ cells per cm^2 from the beginning until the end of the stationary phase. From this microbial density, the average respiration rate of CO_2 was 1.2×10^{12} molecules $cell^{-1}$ h^{-1}, and the average emission of mVOCs was 2.4×10^9 molecules per cell h^{-1}. The mVOC emission rate per cell is reported for the first time and is in the upper range of emission reported previously per gene copy by Adams et al.,[21] where gene copy number is used as a proxy of microbial abundance. At the end of the stationary phase, a starvation/decline phase begins due to either substrate depletion, accumulation of waste products, or insufficient moisture of the media which can be observed from the decline in respiration. The total mVOC emissions continued to increase for approximately 3 h most likely due to liberating the contents of the cells and possible autophagy. The lag of the mVOC decline relative to the decrease in respiration results in enhanced emission factor per cell, but this phase is difficult to quantify due to unsteady biological metabolism which also results in changes in mVOC composition. Consistency in EFs from replicates is no longer expected as the emissions are no longer tied closely to metabolism.

Figure 1d shows the five most abundant volatile metabolites and remaining 410 observed masses ("other"). The composition of the sum of mVOCs in *P. syringae* is dominated by methanol. It is interesting because large methanol emissions have been attributed to the product from the pectin

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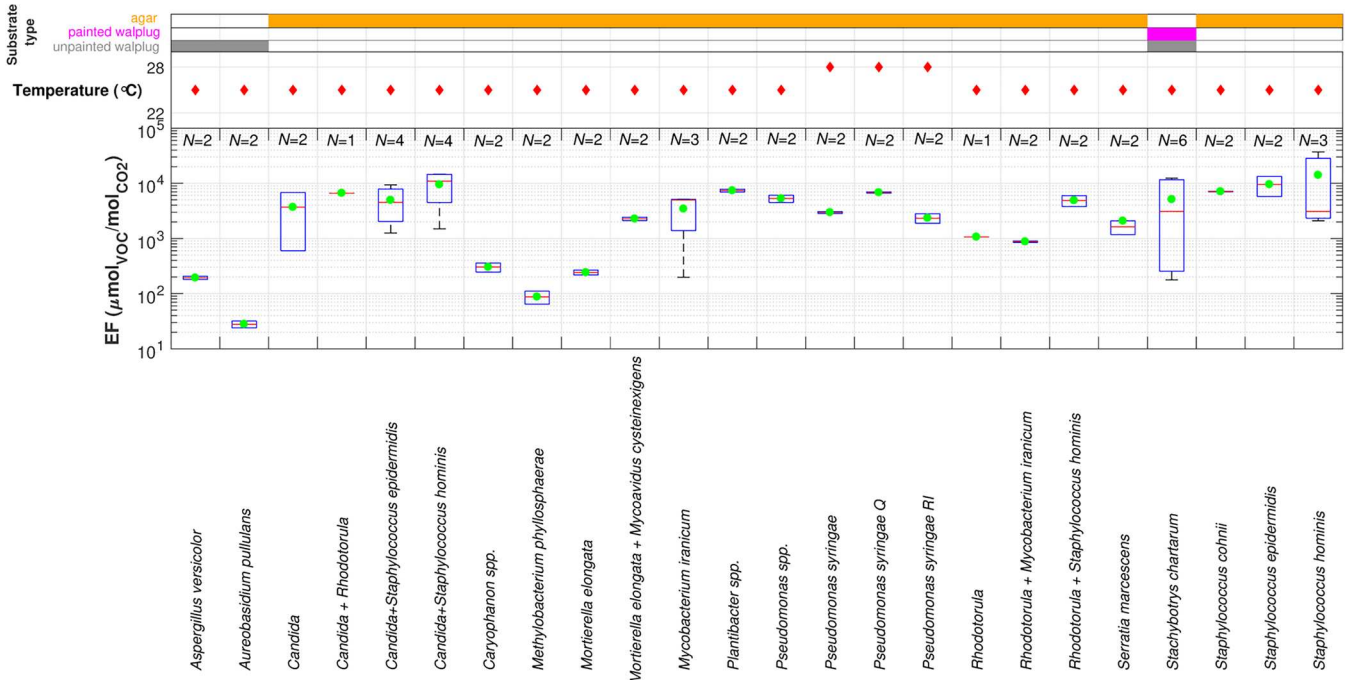

Figure 3. Variability in ΣmVOC emission factors according to microbial species stacked with controlled parameters (temperature, substrate type).

metabolism, which includes pectin methoxylation and/or demethylation of cell walls, as well as pectinolytic activity.[22] However, as the biotrophic pathogen *P. syringae* does not typically exhibit pectinase activity, and pectin is not included in King's B medium, these methanol emissions are probably not related to pectin breakdown.[23] After the end of stationary phase, one can see that less methanol, and more methanethiol, methanediol, and other compounds indicative of oxidation stress and cell breakdown are produced. The fresh uninoculated medium is also a source of VOCs from soluble nutrients, but its composition is different and usually the majority of volatile constituents evaporate within 12 h. However, VOCs such as ethanol, acetaldehyde, benzaldehyde, and relatively small emissions of methanol are frequently found in initial media emissions and they could overlap with the same compounds emitted by microbes. For this reason, it is crucial to measure uninoculated media emissions in parallel to the microbial samples as a control.

Effects of Taxa and Substrate on mVOC Emission Factor. Similar to *P. syringae* analysis, we derived stationary-phase EFs for total and individual mVOCs from a collection of 56 fungal and bacterial strains. A remarkable result is that total mVOC EFs normalized for cell respiration among fungi, bacteria, and their combinations were all within 1 order of magnitude of each other (Figure 2). This is perhaps a surprising similarity given the much higher variability in microbial abundance and absolute emission rates of individual mVOCs, regardless of the variance in the compound composition. However, fungi grown alone exhibited the lowest total mVOC emission strengths on average, compared to bacteria, fungal−bacterial. and fungal−fungal mixed community samples.

Substrate type was also an important factor, with an order of magnitude lower ΣVOC EFs from fungal monocultures grown on unpainted wallboard. An important confounding factor to fungal and bacterial growth is water availability, and this factor is not independent of surface type because of porosity differences resulting in unpainted wallboard being less moist than painted wallboard over time.

The variability in EFs of individual species made up by different strains fed on the same or different substrates is summarized in Figure 3. All the EFs correspond to periods of stationary growth. The incubation temperature was kept at 25 °C (except for *P. syringae* where temperature was 28 °C). As each sample contained liquid water, the water activity in all samples was likely 0.8−1.0 during the stationary phase. The variations in gas-phase relative humidity depend not only on the evaporating water from the wet substrate but also the humidity of the flowing zero air. On average, the measured relative humidity in the chambers was 80 (−20/+10)%, but it is not equivalent to water activity in the substrate, as equilibrium between liquid water in the chamber and water vapor cannot be assumed. However, air relative humidity of less than 70% was associated with lower mVOC emissions. This was the case for one sample of *Stachybotrys chartarum* grown on unpainted wall plugs. This species is known to be particularly dependent on water availability on walls[24] so even a small change in water activity could explain a larger variation of EFs from *S. chartarum*. Another possibility is that this species exhibits a larger variation in the sum of mVOC due to differently expressed secondary metabolism.

Although the variability in ΣmVOC EFs spanned 3 orders of magnitude between individual taxa, in the majority of microbes fed on the same substrate and measured at the same time, the emission factor of the same strain (e.g., *Aspergillus versicolor*) or combined microbial strains (e.g., *Rodotorula* + *Mycobacterium smegmatis*) was consistent among replicates. This suggests that for the same substrate, life stage, and conditions, the emission strength per cell is primarily driven by microbial taxon. The largest intraspecies variabilities were observed when different substrates were used (e.g., *Stachybotrys chartarum*), and/or when water activity was not exactly the same. The only exception was one of the three replicates of *Staphylococcus hominis* that was measured at a different time. It is possible that

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Table 1. Summary of Microbial Taxa Included with Microbial Volatile Sampling Experiments

species	replicates	ID (w/reference)	category (w/ reference)	community structure (B, bacterium; F, fungus)	substrate type/ medium[a]
Aspergillus versicolor	2	VM01US-59[40,41]	building[42]	F	UPWB
Aureobasidium pullulans	2	AA02US-358 (28)	plant[43]	F	UPWB
Candida	2	Personal collection	skin[44,45]	F	MEA
Candida + *Rhodotorula*	1	personal collection	skin	FF	NA
Candida + *Staphylococcus epidermidis*	4	personal collection/ATCC-14990	skin	FB	NA
Candida + *Staphylococcus hominis*	4	personal collection/DSM-103553[46]	skin	FB	NA
Caryophanon spp. H81	2	personal collection	building	B	NA
Methylobacterium phyllosphaerae H73	2	personal collection[46]	plant	B	NA
Mortierella elongata	2	AG77[47]	soil/plants[47]	F	MEA
Mortierella elongata + *Mycoavidus cysteinexigens*	2	AG77[47]	soil/plants[47]	FB	MEA
Mycobacterium iranicum H39	3	DSM-103542[46]	skin	B	NA
Plantibacter spp.	2	DSM-103507[46]	plant	B	NA
Pseudomonas oryzihabitans H72	2	DSM-10350S[46]	plant	B	NA
Pseudomonas syringae B728a	2	personal collection[20]	plant	B	KBA
Pseudomonas syringae Q	2	personal collection[20]	plant	B	KBA
Pseudomonas syringae RI	2	personal collection[20]	plant	B	KBA
Rhodotorula	1	personal collection	skin[45]	F	MEA
Rhodotorula + *Mycobacterium iranicum H39*	2	personal collection/DSM-103542[46]	skin	FB	MEA
Rhodotorula + *Staphylococcus hominis*	2	personal collection/DSM-103553[46]	skin	FB	NA
Serattia marcescens	2	personal collection	building	B	NA
Stachybotrys chartarum	6	AA02US-287[40]	building[42]	B	PWB or UPWB
Staphylococcus cohnii H62	2	DSM-103510[46]	skin	B	NA
Staphylococcus epidermidis	2	ATCC-14990	skin	B	NA
Staphylococcus hominis	3	DSM-103553[46]	skin	B	NA

Uninoculated Blanks and Controls		
	N	% total
agar media blank	9	11.4
painted wallboard blank	2	2.5
unpainted wallboard blank	2	2.5
zero air blank	10	12.7

Microbe Origin		
	N	% total
common to indoor	14	17.7
common to human skin	30	38
common to plant or soil	12	15.2
total	79	100

[a]PWB, painted wallboard; UPWB, unpainted wallboard.

this case might represent differences resulting from a subtle variation in medium composition or due to measurements during a slightly different period of the stationary phase. There may be other factors which we have not evaluated in this study (e.g., pH, VOC uptake) but it may be determined in future studies if or how they can affect mVOC emission profiles.

Chemical Composition in Relation to Microbe Origin. Before addressing variance in group and specific compounds on standardized taxa EFs, we address whether microbial taxa categorized based on origin (dermal, plant, indoor surface), as shown in Table 1, can be broadly characterized by their specific and total emission strengths. We show that bacteria and fungi originating on dermal surfaces dominate the total absolute mVOC EFs, followed by building-associated taxa and plant microbes (Figure 4). The differences were on average within a factor of 2, indicating that the total mVOC emission rate normalized for respiration is not strongly dependent on the source category. The top 50 most abundant ions constituted 94% of the total mVOC EFs comprised by 469 ions. The top three are consistent with methanol, ethanol, and acetone. The remaining ions represent a diverse bouquet of fatty acid associated metabolites, terpenoid compounds, signaling molecules, and other metabolites such as aromatic, nitrogen containing, sulfur containing, or amino acid ester related compounds.

Methanol and methanethiol (CH_4S) are universally emitted by bacteria. The latter compound serves as a more useful generic tracer for bacteria because it has fewer other sources in the indoor environment, whereas methanol is often very abundant indoors due to its relatively low reactivity and ubiquitous sources including human breath and wood products.[25] The fourth and fifth most highly emitted ions are $C_5H_{10}H^+$ and $C_4H_8H^+$. The correlation matrix performed on the entire data set suggests that these ions are not dominated

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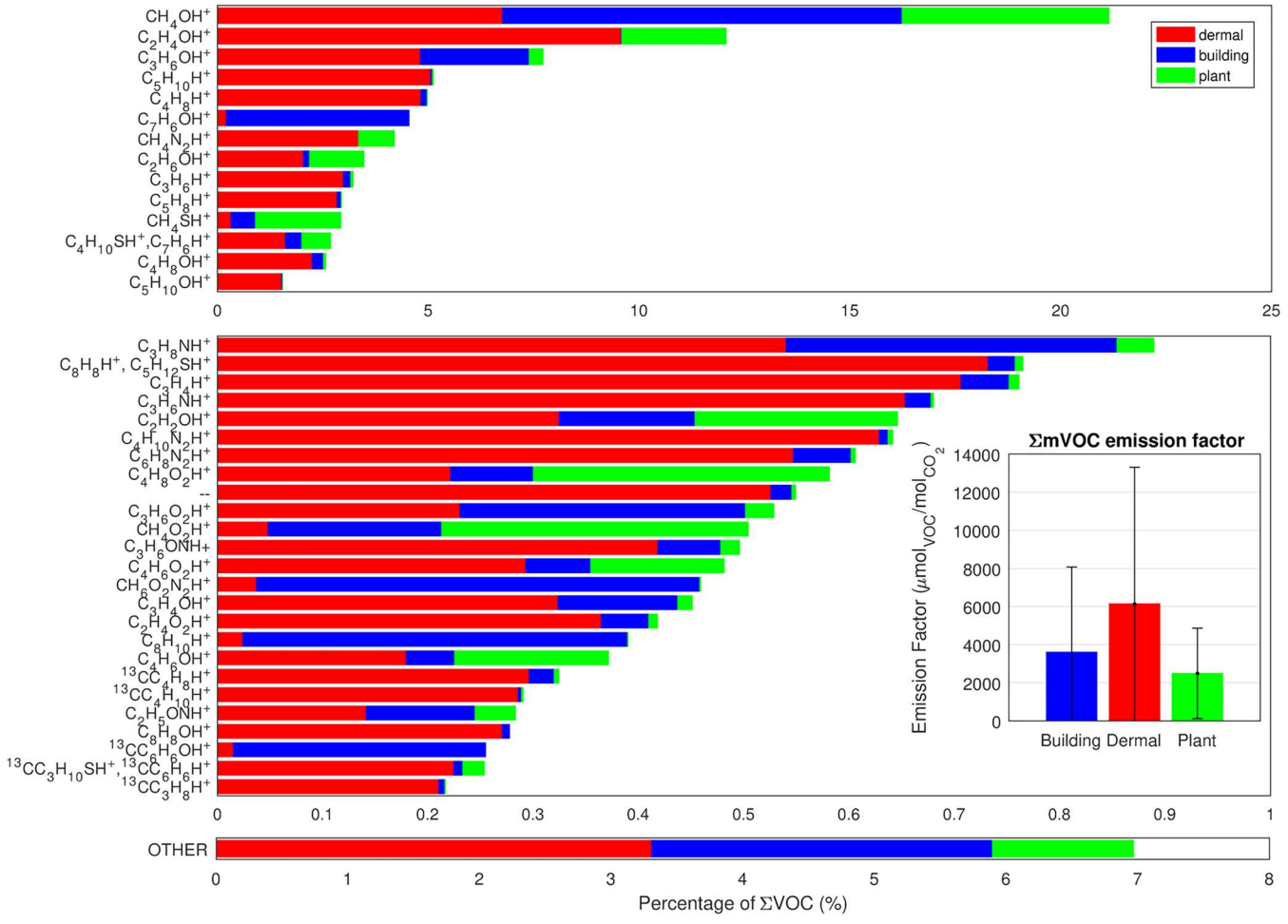

Figure 4. Emission factors averaged for all 56 microbial strains and sorted by abundance. The 39 most abundantly emitted ions presented here constituted 92% of the total mVOC emission factor across taxa, while the remaining 423 ions (other) made up only 7% of the total.

by alkane/alkene parents but are alkyl fragments from higher alcohols, fatty acids, and amino acid esters. These ions were almost completely dominated by dermal types of microbes, although were also emitted by plant and building microbes at much smaller EFs. Interestingly, these ions were also very abundant on biofilms grown in a house (kitchen sink and bathroom tiles).[21] A $C_7H_6OH^+$ ion consistent with benzaldehyde is often present on building materials and was occasionally elevated in the wallboard substrates just after insertion. This is unlike the case for controls where this ion decays usually to background levels within ∼12 h, benzaldehyde production in microbial cultures increased over time, and this particular ion was most abundant in microbes commonly found in buildings. Indoor environments are often noted to contain aromatic compounds from solvents and other anthropogenic sources. Aromatic amino acids (phenylalanine, tyrosine, and tryptophan) constitute about a quarter of plant biomass in large part due to their abundance in the structural biopolymer lignin. Although plants are major producers of aromatic compounds in the atmosphere, they lack the capability to degrade them, unlike microbes which have developed various degradation pathways for utilizing aromatic compounds.[26] The case with dermal microbes is particularly fascinating in terms of how they convert aromatic substrates or synthesize aromatic compounds from phenylalanine. The metabolic strategy microbes take is unique and it has recently been found that catabolism of phenylalanine ($C_9H_{11}NO_2$) and styrene (C_8H_8) proceeds via phenylacetate ($C_8H_8O_2$) in many microbes.[27] We found that the $C_8H_8H^+$ (styrene) and $C_8H_8OH^+$ (acetophenone) ions likely originate from this pathway and were particularly abundant in *Staphylococcus hominis*, a commensal dermal bacterium. Amino acids are not abundant in the gas phase due to their low vapor pressures and high boiling points. However, amino acid esters (e.g., ethyl alaninate) have boiling points approximately 100 °C lower than amino acids (e.g., alanine), and if they are emitted they should be observable by the instrumentation used here. The $C_8H_8O_2$ (phenylacetate or methylbenzoate) ion was correlated with nitrogen-containing ions, some of which could be related to amino acid esters from amino acid degradation and reactions with abundant alcohols (methanol, ethanol).

It seems remarkable that plant-isolated microbes dominate methanethiol production, but apparently not any other metabolite in the top 39 except for methanediol and butyric acid. Methanethiol seems to be a byproduct of the excess substrate for *S*-adenosyl methionine (SAM), an antioxidant protecting cells from the oxidative stress, and its emission is smaller in dermal and building microbes. The abundance of individual VOCs is important for their detection ability in diluted indoor atmospheres but another important feature is compound prevalence across taxa (Supporting Informaiton Figure S3). By taking an emission ratio of abundant and

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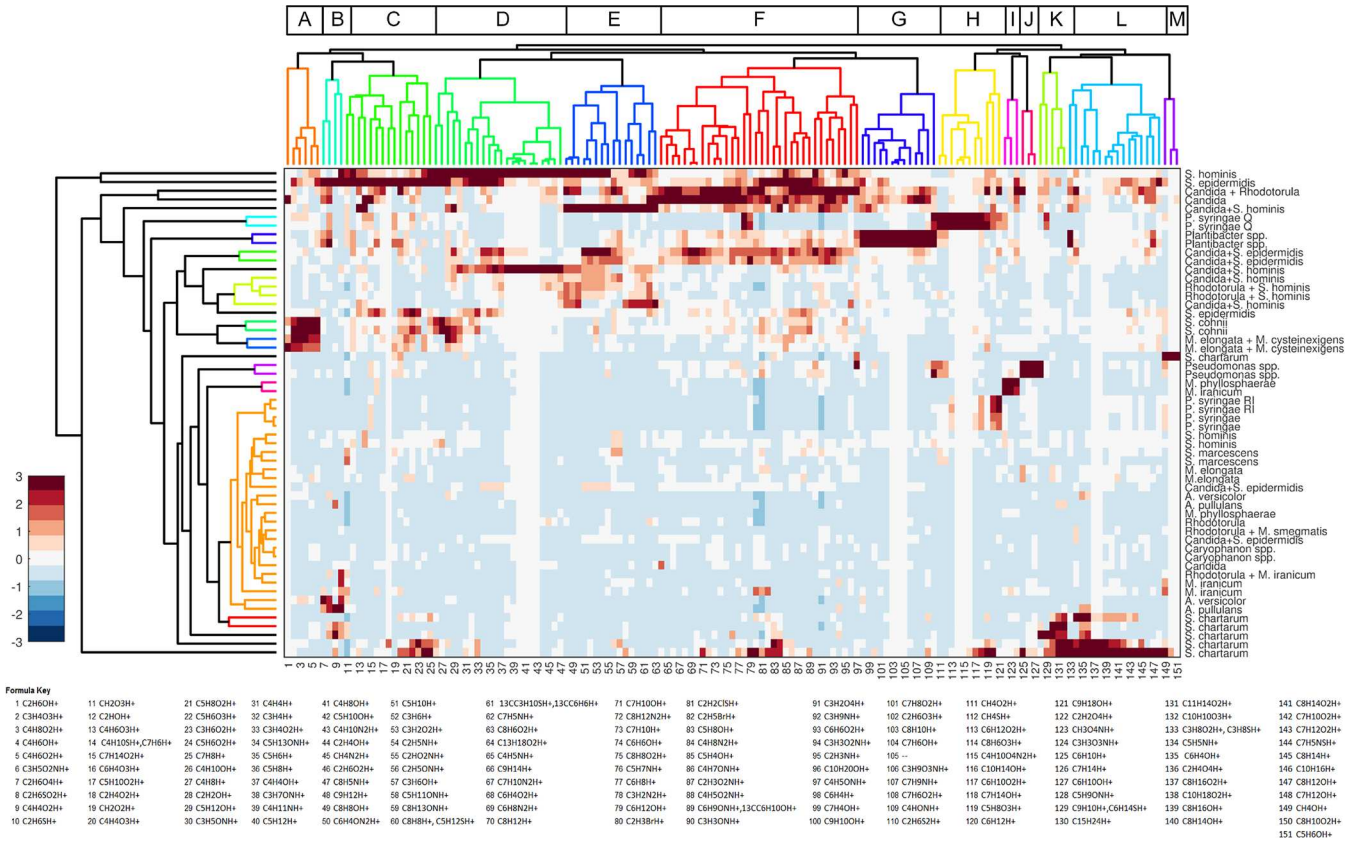

Figure 5. mVOC Dendogram of 151 ions with emission factors >0.1 umol/mol$_{CO2}$.

prevalent mVOCs it is possible to increase information about type of microbes. Specific examples could be the ratios of pyruvic acid to methanethiol, styrene to acetamide, and octenol to styrene, which could be utilized to identify representative microbial species within plant, dermal, and building categories, respectively.

Taxonomic and Chemical Relationships Are Revealed from mVOC Profiles. Although we showed that the life stage and microbial interaction structure has important influence on the magnitude of mVOCs, and to some extent on their composition, the most critical factor explaining the variance seems to be tied to the biological taxon and substrate type. We investigate these relationships using hierarchical dendrograms to infer taxonomical relationships and genomic dissimilarities. The input emission data set constrained to EFs exceeding 0.1 μmol/molCO_2 fed EFs to the clustergram model which standardized 151 ions and used full linkage for 56 microbial strains at 10 levels, elucidating 12 major mVOC groups and grouping microbial taxa according to their chemotype. The cluster patterns show distinct fingerprints. The question of generic and species-specific tracers for mVOCs has been discussed for single compounds, and has been shown to have uncertainties in definitively inferring microbial activity. Dendrogram results (Figure 5) demonstrate that when numerous mVOC ions are measured, the fingerprints provide characteristic clusters which are very sensitive and selective to the taxon and/or interactions of cocultured taxa. Overall, it is clear that the replicates of the same strains were often similar at the first similarity level but this was not always the case, when the substrate was different as for *S. chartarum* due to large differences between possible secondary metabolisms.

The A cluster group is related to pyruvate metabolism which grouped *Staphylococcus cohnii* and *Mortierella elongata* with its bacterial endosymbiont *Mycoavidus cysteinexigens* at eighth level. A characteristic feature of that group of compounds is the production of fatty acids from the pyruvate pathway leading to high butyric acid and butyrate. Group B is clustered with sulfur-containing compounds C_2H_6S consistent with DMS + ethanethiol and $C_2H_6SO_2$ consistent with dimethyl sulfone, and other oxygenated molecules. These compounds were characteristic of dermal microbes and in particular *Mycobacterium iranicum*. This species was grouped with all replicates including *M. iranicum* grown with other combinations. Alhough not grouped together, this fingerprint was encompassed by more broadly emitting *Staphylococcus epidermidis*. Group C is very interesting, as it embraces 2- and 3-oxygen-containing ions consistent with SCFA and hydroxy or keto acids. These metabolites were quite common for various microbes being a generic tracer of bacterial or fungal fatty acid production. Group D was particularly sensitive to *Staphylococcus hominis* and its combinations, and contained compounds related to phenylacetate metabolism including ions consistent with alkyl fragments, styrene, pyruvaldehyde, and acetophenone. Group E comprises chemical compounds emitted predominantly by dermal microbes. It is fascinating to observe microbial combinations of dermal bacteria and fungi (e.g., *Candida* + *S. hominis*, *Candida* + *S. epidermidis*, etc.) grown as cocultures are grouped close to each other but further away from the same individual strains grown as monocultures. It is clear that the interacting microbes manifest different chemotypes although with some degree of overlap in similarities to their monocultures. Many nitrogen-containing compounds in this group possibly come from protein

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degradation. Little is known about the chemistry of bacterial−fungal interactions, and particularly about volatile signaling between microbes. Jones et al.[28] recently showed that fungal VOCs (also nitrogen-containing) affected the exploratory mode of *Streptomyces* development. Looking at time-resolved microbial interactions through mVOC lenses could provide an interesting path forward to enhanced insights.

Fungi were more specifically clustered by the relatively broad compound Group F including some sulfur-containing compounds and unsaturated alcohols and carbonyls typical for fungi. Dermal- and building-associated fungi showed similarities in this group. On the other hand, the next group, G, was specific most strongly to *Plantibacter* spp., a bacterium common to plant galls. The ions characteristic for this group included $C_7H_8O_2$ (consistent with guaiacol) and C_7H_8O (consistent with anisole and/or benzyl alcohol). Three compounds within Group H were unique to *P. syringae* mutant Q$_1$ which lacks the enzymatic capacity to consume pyruvic acid and additional products of the 5-gene pyruvic acid overaccumulation operon regulated by quorum sensing.[20] It is possible that such products are produced by this operon's ferredoxin, heme oxygenase, dihdyropicolinate synthase, peptidase, and aminotransferase putative enzymatic capacities. Groups I and J are fairly small and associated with similar overlapping plant microbes, specifically *P. syringae* and *Methylobacterium phyllospherae* and ions $C_5H_9NOH^+$ (matches ethyloxazoline) and $C_6H_{14}S$ (diisopropylsulfide, hexanethiol, and isomers). The next two groups (K and L) are terpenoid related and unsaturated oxygen compounds, grouping strongly all but one *Stachybotrys chartarum* and less strongly *Candida*. The last group M is unique to one replicate of *Stachybotrys chartarum* in that it had ions consistent with C_5H_6O (cyclopentenone) and $C_8H_{10}O_2$ (tyrosol), which separated it from the previous two neighboring groups, so the K, L, and M embraced all *Stachybotrys* species.

Because the incubation temperature was kept constant during each stationary phase as needed for consistent comparison of emission factors, we have not focused on the specific role of temperature. It is well-known that temperature affects metabolic rates and would be expected to influence the magnitude of mVOC emissions or could lead to death of cells at extreme temperatures. We found that when temperature is decreased, the emissions subside probably because of metabolic rate slowdown and lower volatility of compounds that remain in larger proportion in the liquid phase. In contrast, at high temperature, both the metabolic rate and the amount of volatilized material were larger. We evaluate the effect of temperature separately, using dermal microbes as an example in the SI. These microbes can thrive at body temperature (36.6 °C), but they continue to live on shed skin flakes whose temperature varies with room temperature. Because the enzymes have different temperature optima it is challenging to evaluate the effect of temperature universally for all the microbes.

The findings from this study have broad implications for understanding the significance of microorganism mVOC emissions to the environment. Microbial emission factor chemical phenotypes vary in magnitude and composition as a function of taxa (genera, species, and rarely strains), growth stage, substrate type, symbiotic interaction, and environmental conditions such as temperature and water activity.

On the basis of the stationary phases of 56 microbial cultures from different origins, we report the default total average mVOC emission factor for respiring microbes as 0.4% per molar basis and 1.2% per carbon the basis of respired CO_2. Microbes convert various types of environmental substrates into their main metabolites and in the environment their lifecycles are much more dynamic than in the laboratory, growing and dying as long as there is available substrate and in response to environmental conditions. Because there is a lack of data on microbial emissions to the indoor environment, our results provide a useful starting point for modeling mVOC emissions from microbes. However, the indoor environment is a complex microenvironment with incredibly high microbial diversity, so it represents a general challenge to make a connection from a culture study to the indoor environment. The microbial species were selected to obtain a reasonably representative mix of microbes isolated from building materials, plant-originated, and human originated. Human occupants and outdoor air have been found to be the main contributors of microbial diversity indoors.[29,30] Furthermore, it has been shown that outdoor vegetation has a major contribution to the microbial content of the nearby outdoor air.[31] Our study provides an initial step for future efforts to model microbial VOC emissions in indoor environments, and further studies should focus on measurements of microbiomes directly inside homes. The less abundant metabolites may not be detected when diluted in air but the diverse number of compounds at low concentrations may, in aggregate, add up to a significant amount of carbon, nitrogen, and sulfur. Our findings are in the upper range of emissions reported from indoor biofilms in a recent study by Adams et al.[21] They estimated using a steady-state model that the sum of mVOC concentrations from periodically wetted surfaces in a nonsick house reach tens of ppb. This seems prominent but relatively minor compared to other indoor sources such as emissions from the house materials,[32,33] or emissions from humans.[25] The actual proportion of microbial vs nonmicrobial VOCs in residences will be directly dependent on the microbial densities, surface areas, and air change rates which can vary significantly by residence, occupant activities, and the house's health status. mVOCs may therefore be a small fraction of the other indoor sources in a dry and healthy home but could become dominant in sick homes or where conditions for microbial growth are favorable. We suggest that single-compound tracers of microbial presence cannot typically be specific due to the likelihood of their emissions through common metabolic pathways shared by different species. However, emission ratios of prevalent metabolic mVOCs and the diverse set of metabolites emissions together can provide signatures that can easily be discerned. Microbial emissions are expected in all environments, and the indoor environment is a specific habitat for the human associated microbiome and other microbes for whom the human creates favorable conditions and substrates. Little is known about how the entire indoor microbiome works together on indoor complex surfaces and how it interacts with organic films,[34] or how it interacts with the abundant volatile organics in indoor air.[35] The most abundant metabolites are expected to be significant contributors to indoor VOC compositions but the low-abundance mVOCs may indirectly alter magnitudes of VOC emissions from eukaryotic hosts via chemical interactions. We showed that interacting taxa result in uniquely different chemotypes, other than individual taxa grown in monoculture. Further understanding of microbial interactions in the environment and their mVOCs contributions requires

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interdisciplinary research programs focused on simultaneous measurements of microbial diversity and broad volatility spectrum of chemicals in residences and other environments.

MATERIALS AND METHODS

The goal of the experiments was to obtain time-resolved mVOC emission measurements from representative cultures of different environmental microbial isolates differing in species, genera, and strains grown under controlled temperature conditions in an incubation chamber and to determine factors affecting the composition and magnitude for the full spectrum of emitted mVOCs. We first examined the factors affecting composition and emission rates of mVOCs from individual species to address how mVOC emissions change during exponential, stationary, and senescence growth phases. We then calculated total and compound-specific mVOC emission rates normalized to the respiration rate for different fungal and bacterial taxa grown on different substrates under controlled experimental conditions. Finally, for the consistent periods in the phases of growth, we evaluated the role of other factors such as substrate type, taxon, microbe−microbe interactions (grown as monoculture vs coculture), and environmental conditions (temperature and humidity) affecting the composition and rates of mVOC emissions.

Growth of Microbiological Cultures. Bacterial and fungal strains were loop-inoculated on 60 mm × 15 mm Petri dishes containing either Nutrient Agar (NA, Difco Laboratories, Detroit, MI), Corn Meal Agar (CMA, Sigma-Aldrich, St. Louis, MO), or Malt Extract Agar (MEA, Difco Laboratories, Detroit, MI), either directly from glycerol stocks or from ongoing cultures. Bacteria were incubated at 28 °C for 48 h and fungi at 27 °C for 3−5 days prior to sniffing. Bacterial and fungal cocultures were incubated at 28 °C for 48 h. Uninoculated media served as controls. *Pseudomonas syringae* B728a and mutants were grown at 28 °C in King's B medium (KB) broth[36] with amendment of 15 g of L21 Difco Agar. Preceding the experiment, cultures were washed 3× with 10 mM KPO_4 and inoculated onto KB as lawns at initial density of 40 CFU/cm^2. *Pseudomonas syringae* pv syringae B728a and mutants were as from Scott and Lindow.[20] Briefly, *Pseudomonas syringae Q* refers to a site-directed mutant of *pdhQ*, *pdhT*, and *qrpR*, and *Pseudomonas syringae RI* refers to a site-directed mutant of *ahlI* and *ahlR* encoding synthesis and perception of *N*-(b-Ketocaproyl)-*L*-homoserine lactone, respectively.

Circular plugs of unpainted and painted drywall (0.635 cm, National Gypsum Company, Charlotte, NC) were made using a hole saw drill attachment. Resulting plugs had a diameter of 3.8 cm and were autoclaved prior to inoculation with microbes. Plugs were loop-inoculated from cultures growing on media.

Experimental Design. Measurements were carried out from March 1 to May 1, 2015 in an incubator to which air sampling lines could be directly connected. The approach used a dynamic flow sampling strategy. Eight identical glass jars, equipped with chemically inert gastight Teflon lids and two polyether ether ketone (PEEK) fittings for tubing connections, were used as dynamic chambers. A bundle of sixteen $^1/_{16}$-in. OD PEEK tubing lines was used to connect the chambers through the incubator's wall to an external VICI Silconert 16-port valve (8 incoming, 8 outgoing ports) which was used to divert one of the 8 streams to analytical instrumentation at relatively short intervals (7.5 min), allowing for near-simultaneous time-resolved measurements of 8 samples at a time. Each chamber housed a Petri dish of 28 cm^2 diameter with either a microbial culture or a control. Two types of controls were always included with each 8-jar set: (1) an instrumental control which served as a zero-air blank with nothing but a Petri dish inside and (2) an experimental control in the form of uninoculated substrate (agar medium, painted or unpainted wallplug). The purpose of using instrumental control was to account for potential trace impurities in the system and allow for accurate instrument background subtraction. The experimental control was designated to discriminate between microbial and nonmicrobial VOC emissions. A typical set of experiments contained 2 different strains in duplicate, but 3 replicate experiments were also performed. Occasionally, only a single replicate was used as a reference for some species (see Table 1 for the summary of microbial taxa and replicates). The two types of controls, either in 1 or 2 replicates, were always included. The zero-air generator supplied dry VOC-free air part of which was diverted through a glass bubbler filled with deionized and sterilized water at a controlled flow rate to set the humidity in the sample flow at desired levels. The enrichment of air in water vapor decreases the rate of evaporation of liquid water from the samples, prolonging the time of high water activity. The humid VOC-free air (zero air) was supplying each chamber at 133 sccm of continuous flow delivered via $^1/_{16}$-in. PEEK tube extended to the bottom of the chamber to distribute the air without causing aeraulic stress to the culture, and to prevent aerosolizing low-volatility compounds.[37] To ensure that only gas-phase compounds were sampled and to prevent particles from entering the instrument, a 1-μm-pore Teflon filter was inserted into each chamber's outflow. The flow rate of zero air through the chamber led to 1 full air exchange per ∼3.5 min and ensured steady-state VOC concentrations in an optimal detection range for the vast majority of emitted VOCs from a typical microbial culture. The flow-through configuration used here has a significant benefit over a dead-ended valve system because the switching is seamless and avoids spikes or residues from previous samples. A detailed flow configuration schematic is presented in the Supporting Information.

VOC Measurements. Time-resolved composition and abundance of volatile organic compounds were measured using a proton transfer reaction time-of-flight mass spectrometer (PTRTOFMS; PTR-TOF 8000, IONICON Analytik GmbH). The PTRTOFMS recorded the mass spectrum as mass-to-charge ratios (m/z) of 1.000−500.000 at a rate of 1 Hz, using H_3O^+ primary reagent ion. H_3O^+ mode allows for soft chemical ionization which is highly sensitive to all those VOC compounds whose proton affinity is higher than that of water, which includes the vast majority of VOCs. The instrument is particularly sensitive to benzenoid compounds, the majority of unsaturated linear, branched, and cyclic hydrocarbons, oxygentated hydrocarbons, alcohols, aldehydes, ketones, acids, lactones, esters, cyclic and heterocyclic compounds, amides, sulfides, thiols, ethers, and many other volatile and semivolatile compounds whose vapor pressure is sufficiently high to be present in the gas phase. There are some compounds that are more challenging to detect, and they include organic nitrates, organosulfates, phosphates, and some amines which may be too sticky to get through the inlet. Short *n*-alkanes have proton affinities lower than water and are therefore not ionized efficiently by H_3O^+, although their detection is still possible via ionization by residual O_2^+/NO^+ ions but since these ions are only present at trace level (∼1−

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3% of H_3O^+) the sensitivity to *n*-alkanes is ∼2 orders of magnitude lower than for other VOCs. Short-chain alkanes therefore do not typically affect the total VOC magnitude reported here unless their abundance is extremely high. Because the ions consistent with *n*-alkanes can also represent alkyl moieties of esters, or larger molecules, these ions should be included to the full total VOC budget. The instrument had a zero-air measurement performed every hour which was included as part of blank jar measurement. The instrument's conditions and settings were kept constant throughout the campaign. The detector relative transmission was verified using multicomponent VOC gas standard mixtures, which included a wide range of VOCs. In addition, calibration on two specifically designed gas mixtures prepared by Apel-Riemer which contained many typical microbial VOCs, such as 1-octen-3-ol, furfural, methional, pentylthiophene, indole, acetophenone, or crotonolactone was performed to verify the responses to these types of molecules. A detailed composition of calibration standards is shown in SI Table S1.

Data Analysis. Raw mass spectra from the PTRTOFMS were processed into counts per second (cps) and raw concentrations (ppb) using a ptrwid software[38] operated in Interactive Data Language (Research Systems, Boulder, CO). The chemical formula matching and compound attribution has been done according to the closest match (typically within 1−2 mDa) of the measured mass with the exact molecular formula mass and subsequently by a series of validation steps such as a correlation matrix on total and specific time segments, isotopic signatures, literature search and various tools (e.g., Chemspider), and the mVOC literature compendium database.[39] The total number of detected ions unified across all measurements was 805. A combination of average abundance filter of 100 ppq (10^{-13}), a filter of known internal ions (primary ions, water clusters, ions source impurities), and other QC/QA procedures such as visual inspection of signal quality was performed and retained 462 ions which passed all the quality criteria. Further information on QC/QA details are included in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b00806.

Effect of Incubation Temperature on Emission Rate in Dermal Bacteria. Effect of Substrate on Secondary Metabolism in *Stachybotrys chartarum*. Prevalence and Specificity of mVOCs. Supplementary Methods. Tables of ions and emission factors. Figure S1. Effect of incubation temperature on ΣVOC emission rate in dermal bacteria where temperature ramp was used (backward and forward). Figure S2. Example mVOC emission dynamics from fungi fed on painted and unpainted wall plugs. Figure S3. mVOC prevalence distribution across taxa. Figure S4. Experimental setup. Table S1. Gas standard mixtures used to calibrate instrument transmission and response to common mVOCs and other compounds. Table S2. Table of ions and mVOC emission factors (μmol/molCO_2). Skin-microbe category. Table S3. Table of ions and mVOC emission factors (μmol/molCO_2). Plant-microbe category. Table S4. Table of ions and mVOC emission factors (μmol/molCO_2). Building-microbe category. (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Salonen, H.; Lappalainen, S.; Lindroos, O.; Harju, R.; Reijula, K. Fungi and bacteria in mould-damaged and non-damaged office environments in a subarctic climate. *Atmos. Environ.* **2007**, *41* (32), 6797−6807.

(2) Miller, M. B.; Bassler, B. L. Quorum sensing in bacteria. *Annu. Rev. Microbiol.* **2001**, *55* (1), 165−199.

(3) Ditengou, F. A.; Müller, A.; Rosenkranz, M.; Felten, J.; Lasok, H.; van Doorn, M. M.; Legué, V.; Palme, K.; Schnitzler, J.-P.; Polle, A. Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. *Nat. Commun.* **2015**, *6*, 7279.

(4) Bönisch, U.; Böhme, A.; Kohajda, T.; Mögel, I.; Schütze, N.; von Bergen, M.; Simon, J. C.; Lehmann, I.; Polte, T. Volatile organic compounds enhance allergic airway inflammation in an experimental mouse model. *PLoS One* **2012**, *7* (7), e39817.

(5) Nurmatov, U. B.; Tagiyeva, N.; Semple, S.; Devereux, G.; Sheikh, A. Volatile organic compounds and risk of asthma and allergy: a systematic review. *Eur. Respir. Rev.* **2015**, *24* (135), 92−101.

(6) Korpi, A.; Järnberg, J.; Pasanen, A.-L. Microbial Volatile Organic Compounds. *Crit. Rev. Toxicol.* **2009**, *39* (2), 139−193.

(7) Sahlberg, B.; Gunnbjörnsdottir, M.; Soon, A.; Jogi, R.; Gislason, T.; Wieslander, G.; Janson, C.; Norback, D. Airborne molds and bacteria, microbial volatile organic compounds (MVOC), plasticizers and formaldehyde in dwellings in three North European cities in relation to sick building syndrome (SBS). *Sci. Total Environ.* **2013**, *444*, 433−440.

(8) Malta-Vacas, J.; Viegas, S.; Sabino, R.; Viegas, C. Fungal and Microbial Volatile Organic Compounds Exposure Assessment in a Waste Sorting Plant. *J. Toxicol. Environ. Health, Part A* **2012**, *75* (22−23), 1410−1417.

(9) Domingo, J. L.; Nadal, M. Domestic waste composting facilities: A review of human health risks. *Environ. Int.* **2009**, *35* (2), 382−389.

(10) Sunesson, A.; Vaes, W.; Nilsson, C.; Blomquist, G.; Andersson, B.; Carlson, R. Identification of volatile metabolites from five fungal species cultivated on two media. *Appl. Environ. Microbiol.* **1995**, *61* (8), 2911−2918.

(11) Bennett, J. W.; Inamdar, A. A. Are Some Fungal Volatile Organic Compounds (VOCs) Mycotoxins? *Toxins* **2015**, *7* (9), 3785−3804.
(12) Inamdar, A. A.; Hossain, M. M.; Bernstein, A. I.; Miller, G. W.; Richardson, J. R.; Bennett, J. W. Fungal-derived semiochemical 1-octen-3-ol disrupts dopamine packaging and causes neurodegeneration. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (48), 19561−19566.
(13) Hospodsky, D.; Qian, J.; Nazaroff, W. W.; Yamamoto, N.; Bibby, K.; Rismani-Yazdi, H.; Peccia, J. Human Occupancy as a Source of Indoor Airborne Bacteria. *PLoS One* **2012**, *7* (4), e34867.
(14) Grice, E. A.; Kong, H. H.; Conlan, S.; Deming, C. B.; Davis, J.; Young, A. C.; Bouffard, G. G.; Blakesley, R. W.; Murray, P. R.; Green, E. D.; Turner, M. L.; Segre, J. A. Topographical and Temporal Diversity of the Human Skin Microbiome. *Science* **2009**, *324* (5931), 1190−1192.
(15) Grice, E. A.; Segre, J. A. The skin microbiome. *Nat. Rev. Microbiol.* **2011**, *9* (4), 244−253.
(16) Brandwein, M.; Steinberg, D.; Meshner, S. Microbial biofilms and the human skin microbiome. *NPJ. Biofilms Microbiomes* **2016**, *2* (1), 3.
(17) Naegele, A.; Reboux, G.; Vacheyrou, M.; Valot, B.; Millon, L.; Roussel, S. Microbiological consequences of indoor composting. *Indoor air* **2016**, *26* (4), 605−613.
(18) Park, J.-H.; Goldstein, A. H.; Timkovsky, J.; Fares, S.; Weber, R.; Karlik, J.; Holzinger, R. Active Atmosphere-Ecosystem Exchange of the Vast Majority of Detected Volatile Organic Compounds. *Science* **2013**, *341* (6146), 643−647.
(19) Nyström, T. Stationary-phase physiology. *Annu. Rev. Microbiol.* **2004**, *58*, 161−181.
(20) Scott, R. A.; Lindow, S. E. Transcriptional control of quorum sensing and associated metabolic interactions in Pseudomonas syringae strain B728a. *Mol. Microbiol.* **2016**, *99* (6), 1080−1098.
(21) Adams, R. I.; Lymperopoulou, D. S.; Misztal, P. K.; De Cassia Pessotti, R.; Behie, S. W.; Tian, Y.; Goldstein, A. H.; Lindow, S. E.; Nazaroff, W. W.; Taylor, J. W.; Traxler, M. F.; Bruns, T. D. , Microbes and associated soluble and volatile chemicals on periodically wet household surfaces. *Microbiome* **2017**, *5* (1), 128.
(22) Volcani, Z. Bacterial Soft Rot of Avocado Fruit. *Nature* **1954**, *174* (4430), 604−605.
(23) Ivanović, Ž.; Perović, T.; Popović, T.; Blagojević, J.; Trkulja, N.; Hrnčić, S. Characterization of Pseudomonas syringae pv. syringae, Causal Agent of Citrus Blast of Mandarin in Montenegro. *Plant Pathol. J.* **2017**, *33* (1), 21.
(24) Boutin-Forzano, S.; Charpin-Kadouch, C.; Chabbi, S.; Bennedjai, N.; Dumon, H.; Charpin, D. Wall relative humidity: a simple and reliable index for predicting Stachybotrys chartarum infestation in dwellings. *Indoor Air* **2004**, *14* (3), 196−199.
(25) Tang, X.; Misztal, P. K.; Nazaroff, W. W.; Goldstein, A. H. Volatile Organic Compound Emissions from Humans Indoors. *Environ. Sci. Technol.* **2016**, *50* (23), 12686−12694.
(26) Fuchs, G.; Boll, M.; Heider, J. Microbial degradation of aromatic compounds — from one strategy to four. *Nat. Rev. Microbiol.* **2011**, *9* (11), 803−816.
(27) Teufel, R.; Mascaraque, V.; Ismail, W.; Voss, M.; Perera, J.; Eisenreich, W.; Haehnel, W.; Fuchs, G. Bacterial phenylalanine and phenylacetate catabolic pathway revealed. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107* (32), 14390−14395.
(28) Jones, S. E.; Ho, L.; Rees, C. A.; Hill, J. E.; Nodwell, J. R.; Elliot, M. A. Streptomyces exploration is triggered by fungal interactions and volatile signals. *eLife* **2017**, *6*, e21738.
(29) Miletto, M.; Lindow, S. E. Relative and contextual contribution of different sources to the composition and abundance of indoor air bacteria in residences. *Microbiome* **2015**, *3* (1), 61.
(30) Meadow, J. F.; Altrichter, A. E.; Kembel, S. W.; Kline, J.; Mhuireach, G.; Moriyama, M.; Northcutt, D.; O'Connor, T. K.; Womack, A. M.; Brown, G.; et al. Indoor airborne bacterial communities are influenced by ventilation, occupancy, and outdoor air source. *Indoor Air* **2014**, *24* (1), 41−48.
(31) Lymperopoulou, D. S.; Adams, R. I.; Lindow, S. E. Contribution of vegetation to the microbial composition of nearby outdoor air. *Appl. Environ. Microbiol.* **2016**, *82* (13), 3822−3833.
(32) Hodgson, A. T.; Nabinger, S. J.; Persily, A. K. *Volatile Organic Compound Concentrations and Emission Rates Measured over One Year in a New Manufactured House*; LBNL-56272; Lawrence Berkeley National Lab (LBNL): Berkeley, CA, 2004; 34 pp.
(33) Wu, X.; Apte, M. G.; Maddalena, R.; Bennett, D. H. Volatile Organic Compounds in Small- and Medium-Sized Commercial Buildings in California. *Environ. Sci. Technol.* **2011**, *45* (20), 9075−9083.
(34) Weschler, C. J.; Nazaroff, W. W. Growth of organic films on indoor surfaces. *Indoor air* **2017**, *27*, 1101−1112.
(35) Salthammer, T.; Zhang, Y.; Mo, J.; Koch, H. M.; Weschler, C. J. Assessing human exposure to organic pollutants in the indoor environment. *Angew. Chem., Int. Ed.* **2018**, DOI: 10.1002/anie.201711023.
(36) King, E. O.; Ward, M. K.; Raney, D. E. Two simple media for the demonstration of pyocyanin and fluorescin. *J. Lab. Clin. Med.* **1954**, *44* (2), 301−307.
(37) Aleksic, B.; Draghi, M.; Ritoux, S.; Bailly, S.; Lacroix, M.; Oswald, I. P.; Bailly, J.-D.; Robine, E. Aerosolization of mycotoxins after growth of toxinogenic fungi on wallpaper. *Appl. Environ. Microbiol.* **2017**, *83* (16), e01001−17.
(38) Holzinger, R. PTRwid: A new widget tool for processing PTR-TOF-MS data. *Atmos. Meas. Tech.* **2015**, *8* (9), 3903−3922.
(39) Lemfack, M. C.; Nickel, J.; Dunkel, M.; Preissner, R.; Piechulla, B. mVOC: a database of microbial volatiles. *Nucleic Acids Res.* **2014**, *42* (D1), D744−D748.
(40) Amend, A.; Samson, R.; Seifert, K.; Bruns, T. Deep sequencing reveals diverse and geographically structured assemblages of fungi in indoor dust. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 13748−13753.
(41) Visagie, C.; Hirooka, Y.; Tanney, J.; Whitfield, E.; Mwange, K.; Meijer, M.; Amend, A.; Seifert, K.; Samson, R. Aspergillus, Penicillium and Talaromyces isolated from house dust samples collected around the world. *Stud. Mycol.* **2014**, *78*, 63−139.
(42) Flannigan, B.; Samson, R. A.; Miller, J. D. *Microorganisms in Home and Indoor Work Environments: Diversity, Health Impacts, Investigation and Control*; CRC Press, 2016.
(43) McGrath, M. J.; Andrews, J. H. Temporal changes in microscale colonization of the phylloplane by Aureobasidium pullulans. *Appl. Environ. Microbiol.* **2006**, *72* (9), 6234−6241.
(44) Roth, R. R.; James, W. D. Microbial ecology of the skin. *Annu. Rev. Microbiol.* **1988**, *42* (1), 441−464.
(45) Findley, K.; Oh, J.; Yang, J.; Conlan, S.; Deming, C.; Meyer, J. A.; Schoenfeld, D.; Nomicos, E.; Park, M.; Kong, H. H.; et al. Topographic diversity of fungal and bacterial communities in human skin. *Nature* **2013**, *498* (7454), 367.
(46) Lymperopoulou, D. S.; Coil, D. A.; Schichnes, D.; Lindow, S. E.; Jospin, G.; Eisen, J. A.; Adams, R. I. Draft genome sequences of eight bacteria isolated from the indoor environment: Staphylococcus capitis strain H36, S. capitis strain H65, S. cohnii strain H62, S. hominis strain H69, Microbacterium sp. strain H83, Mycobacterium iranicum strain H39, Plantibacter sp. strain H53, and Pseudomonas oryzihabitans strain H72. *Stand. Genomic Sci.* **2017**, *12* (1), 17.
(47) Uehling, J.; Gryganskyi, A.; Hameed, K.; Tschaplinski, T.; Misztal, P. K.; Wu, S.; Desirò, A.; Vande Pol, N.; Du, Z.; Zienkiewicz, A.; Zienkiewicz, K.; Morin, E.; Tisserant, E.; Splivallo, R.; Hainaut, M.; Henrissat, B.; Ohm, R.; Kuo, A.; Yan, J.; Lipzen, A.; Nolan, M.; LaButti, K.; Barry, K.; Goldstein, A. H.; Labbé, J.; Schadt, C.; Tuskan, G.; Grigoriev, I.; Martin, F.; Vilgalys, R.; Bonito, G. Comparative genomics of Mortierella elongata and its bacterial endosymbiont Mycoavidus cysteinexigens. *Environ. Microbiol.* **2017**, *19* (8), 2964−2983.

DOI: 10.1021/acs.est.8b00806
Environ. Sci. Technol. 2018, 52, 8272−8282