



Occupants contribute to pathogens and probiotics in indoor environments

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ABSTRACT

A majority of airborne bacteria in indoor environments are associated with occupants, among which pathogens and probiotics are of particular concern due to their health impacts. Occupants are an important source of indoor bacteria. However, the role of occupants in shaping the distributions of pathogens and probiotics remains to be fully understood. To this end, we collected total suspended particulates from six indoor (office, dormitory, and apartment) and outdoor (crossroad, doorway, and playground) environments, followed by identifying the pathogens and probiotics at the species level via the nanopore sequencing technology. Furthermore, the emission rate of the total bacteria, pathogens (*Staphylococcus aureus*), and probiotics (*Lactobacillus*) in the indoor environments were estimated via the culture-dependent method. Our results showed that the indoor/outdoor ratio of relative abundance for pathogens was 2 while that for probiotics was 8. The emission rates of total bacteria, *Staphylococcus aureus* (pathogen), and *Lactobacillus* (probiotics) from the occupants were 23380, 1508, and 3994 CFU·person⁻¹·h⁻¹, respectively. Notably, the emission rate of *Lactobacillus* was highest in the female lavatory compared to those in other environments, suggesting females as an important source of indoor probiotics. Our study offers insights into the occupant contribution to the airborne pathogens and probiotics, and the bacteria-associated health impacts in indoor environments.

1. Introduction

Indoor environments harbor a large community of bacteria that differs significantly from those in outdoor environments in terms of concentration and composition [1]. For many indoor environments, such as residences [2], schools [3], and offices [4], when occupants were present, the concentrations of culturable bacteria were higher than those in outdoor environments. So were the diversity and richness of bacteria [5]. However, Zhou et al. reported the opposite cases where the bacterial concentration and diversity (termed by the Shannon indices) in the indoor environments, when not occupied, were lower than those in the outdoor environment [6]. It appears that occupants play a pivotal role in shaping the bacterial community in indoor environments.

Indoor bacteria originate from different pathways, such as skin shedding [7], respiratory droplets [8], and feces excretion [9]. As the largest organ of the human body, the skin is home to millions of bacteria that include both pathogens and probiotics [10]. Pathogens, such as *Staphylococcus* and *Propionibacterium*, prefer to inhabit the moist

popliteal crease [7,10] while *Lactobacillus*, a typical probiotic, is mostly found in *Labia minora* [11]. These bacteria could disperse into the indoor environment via skin-to-surface contact and/or direct shedding of biological particles [1]. The respiratory tract is another habitat for pathogenic bacteria, e.g., *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* [12], which can be emitted into the indoor environment by respiratory droplets when occupants cough, sneeze or speak [8]. Gut bacteria is a substantial part of the human microbiome and capable of transferring into the environment by feces [9]. Probiotics, such as *Bifidobacterium* [9] and *Lactobacillus* [13] are commonly found in human feces.

Some researchers have quantified the total bacterial emission from occupants to indoor environments [14–16]. Qian et al. found the emission rate of the total bacteria in a university classroom was $\sim 10^7$ genome copies·person⁻¹·h⁻¹ [14], in line with the order of magnitude of the emission rates reported by Hospodsky et al. for six occupied children's classrooms [15]. Scheff et al. found that the emission rate of culturable total bacteria was 13620 CFU·person⁻¹·h⁻¹ in a middle

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school [16]. However, few studies have investigated the emission rates for pathogens and probiotics that directly affect human health.

To properly characterize the occupant contribution to the bacterial communities for pathogens and probiotics in indoor environments, the culture-dependent and -independent methods were both employed in our study. The culture-independent nanopore sequencing technology was used to identify the distribution of the pathogens and probiotics at the species level while the culture-dependent method was used to quantify the emission rates of pathogens and probiotics originating from occupants. Six types of indoor and outdoor environments were selected to cover various occupant activities. Our study aims to 1) characterize the pathogens and probiotics at the species level in indoor and outdoor environments 2) conclude the potential health effect of the identified pathogens and probiotics 3) quantify the emission rates of pathogens and probiotics from occupants.

2. Materials and methods

2.1. Sample collection for sequencing

Total suspended particulates (TSP) were sampled by an impact sampler (Laoshan Application, Qingdao, China) with the fiber filter ($\Phi 80$ mm) loaded on [17]. The flow rate of the sampler is 100 L min^{-1} , and the sampling height is 1.5 m from the ground. Sampling sites include 3 indoor environments, i.e., office, dormitory, and apartment, and 3 outdoor environments, i.e., crossroad, doorway, and playground. The sampling was conducted from 8:00 to 16:00 for 3 days in January 2021 at each site (Table S1). Thus, the volume of the air flowing through the sampler for each sample was 48 m^3 . The particulates were scraped from all of the filters with a sterilized lancet. All of the particulates were frozen at -20°C before treatments.

2.2. DNA extraction and quantitation

DNeasy PowerSoil Kit (QIAGEN, Germany) was used to extract the DNA from particulates. The extraction steps were in strict accordance with the manufacturer's instructions [18]. For each sampling site, samples collected for 3 days were mixed to obtain enough DNA for the following analyses. The concentration of total bacteria was quantified by the quantitative Polymerase Chain Reaction (qPCR) method with the primers of 341F/534R in the V3 region of 16S rRNA genes [19,20]. More details of the qPCR method were described in the previous study [18]. Two kinds of negative controls were included in the DNA extraction and qPCR processes: 1) unexposed filters were treated simultaneously with exposed filters; 2) DNase/RNase-Free water was contained in all the experimental steps. After the qPCR, the results of these controls were negative, indicating no contamination in our experiments [21]. The positive control, i.e. bacterial genomic DNA with known concentration was used to ensure the experiment was inerrant.

2.3. Nanopore sequencing

The full-length of 16S rRNA genes with primers of 27F/1492R for bacteria were measured by using Oxford Nanopore MinION portable sequencer [22]. DNA was prepared using a Nanopore SQK-LSK109 kit under the manufacturer's instructions [23]. The final DNA was quantified by using Qubit (V3.0) to ensure at least 500 ng of DNA remained [24]. Flow cells (Oxford Nanopore FLO-MIN106) were fitted onto the MinION device to conduct the sequencing process. The gene sequences run on a Linux system for 4 h through the MinKnow software (V2.2). Finally, the DNA sequences were read in FAST5 format and then converted into FASTQ format by Guppy (V4.2.2). More details were shown in previous studies [22,25].

2.4. Phylogenetic analyses

After sequencing, the generated reads were analyzed by using NanoPlot (V1.30.1) [26]. The Greengenes (V13.5) database was used to identify the bacterial phylotypes. Certain species were confirmed by the clustering method with a 99% similarity threshold [27]. Then, we used the FAPROTAX database (V1.2.4) and cited previous studies to identify the potential pathogens and probiotics [20,28–30].

2.5. Emission rate estimation

The culture-dependent method was used to estimate the bacterial emission rate from occupants in three types of rooms: working place, bedroom, and lavatory. The dominant species *S. aureus* was chosen as the indicator for the various pathogenic species. The genus *Lactobacillus* with the most diverse probiotic species was selected to represent the probiotics. The emission rates of total bacteria, *S. aureus*, and *Lactobacillus* from occupants were calculated by Equation (1) developed by Qian et al. [14].

$$C = fC_{out} + \frac{NE}{Q + KV} \quad (1)$$

Where C is the average concentration ($\text{CFU} \cdot \text{m}^{-3}$) of total bacteria, *Staphylococcus aureus*, and *Lactobacillus* in the indoor environment; C_{out} is the corresponding average concentration in the outdoor environment which was measured simultaneously with the indoor sampling; f is the indoor-outdoor ratio of the average concentration when the room is unoccupied; N is the average number of occupants; E is the emission rate ($\text{CFU} \cdot \text{person}^{-1} \cdot \text{h}^{-1}$) for total bacteria, *S. aureus*, and *Lactobacillus*, Q is the air exchange rate ($\text{m}^3 \cdot \text{h}^{-1}$); V is the room volume (m^3), and k is the size-specific deposition-rate coefficient for total bacteria, *S. aureus*, and *Lactobacillus* (per h). The air exchange rate was determined by the tracer (CO_2) decay method [31]. The deposition-rate coefficients (k) of total bacteria, *S. aureus*, and *Lactobacillus* were 8.6, 0.31, and 0.79 respectively [14,32,33].

The bacterial concentration was calculated by counting the colonies on plates with specific agar mediums. The sampling plates were placed at the center of each room at the height of 0.8–1 m. At each sampling site, after 30-min sterilization by a UV lamp, the sampling was conducted in the unoccupied condition for 30 min. Then occupants entered the rooms and stayed for about 8 h before the commencement of the sampling in the occupied condition which also lasted for 30 min. Fig. 1 shows the detailed schedules for all samplings. Outdoor sampling was conducted simultaneously. The sampling at each site was repeated 3 times to give an average concentration. It was natural ventilation for all rooms and the windows were open throughout the experiments. There are no centralized air conditioners, ceiling fans, pedestal fans, or exhaust fans in the rooms. All rooms are free of pets. The number and gender of the occupants were recorded. More details of the experimental conditions were shown in Table S2.

After the sampling, bacteria loaded on different mediums were cultured in proper conditions. *S. aureus* was cultured on Baird-Parker (BP) agar medium at 37°C for 24 h [34]. *Lactobacillus* was cultured on the Man Rogosa Sharpe (MRS) agar medium at 37°C for 24 h [35]. Total bacteria were cultured on Tryptic Soy agar (TSA) medium at 30°C for 24 h [36]. After the culturing, we counted the number of bacterial colonies on the medium plates.

2.6. Statistics analyses

The bacterial richness (observed species, Chao1, abundance-based coverage estimator (ACE)), and diversity (Shannon and Simpson indices) were calculated by using the packages ("vegan" and "picante") in the R environment (V4.1.0) [37,38]. The feature species leading to significant differences in the bacterial communities were determined by

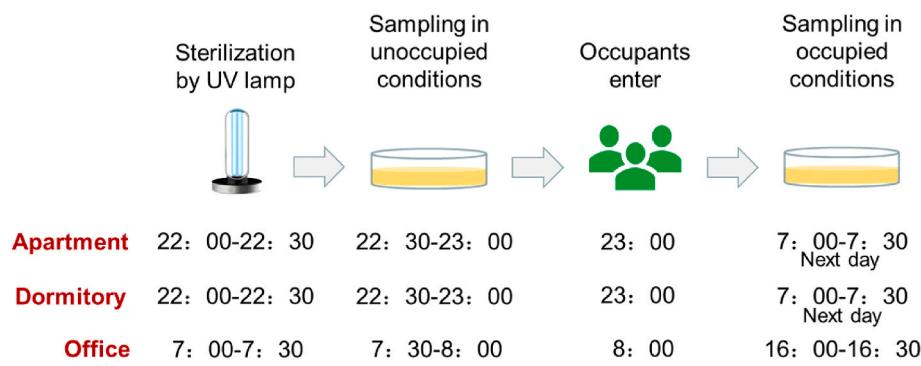


Fig. 1. The sampling schedule of culture-dependent method in one day.

the linear discriminant analysis (LDA) Effect Size (LEfSe) [39]. The distribution and relevant diseases of pathogens and probiotics were visualized by the heatmap and alluvial diagram in the Origin (V2021) software.

3. Results and discussion

3.1. The comparison of indoor and outdoor bacterial communities

Samples were collected from three types of indoor environments (office, dormitory, and apartment) and three types of outdoor environments (crossroad, doorway, and playground). A total of 1869 OTUs and 436 species were observed in all the samples. Except for the unclassified bacterial taxonomy, the average number of classified species in indoor environments was 219 while that for outdoor environments was 181 (Table S3). Based on the qPCR results, the average concentration of total indoor bacteria ($4.42 \times 10^5 \pm 2.95 \times 10^5$ copies·m⁻³ air) was ~20 times than that of total outdoor bacteria ($2.25 \times 10^4 \pm 1.01 \times 10^4$ copies·m⁻³ air) (Table S4). The result was in line with the previous studies showing the bacterial concentration in occupied indoor environments was higher than that in outdoor environments [2,3]. The top 5 species ranked by average relative abundance in indoor environments were *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus sciuri*, and *Janthinobacterium lividum*, and they are all associated with specific diseases (Fig. 2a). The top 5 species in outdoor environments were *Psychrobacter pulmonis*, *Janthinobacterium lividum*,

Serratia marcescens, *Clostridium perfringens*, and *Salmonella enterica*, and 4 of them are pathogens. (Fig. 2a).

The features species which are the major species differentiating different bacteria communities were determined by the linear discriminant analysis Effect Size (LEfSe) (Fig. 2b). In total, 21 feature species were identified, among which only 5 species were more abundant (higher LDA scores) in outdoor environments while the other 16 species were more abundant in indoor environments. Half of the identified feature species (11 out of 21) were regarded as potential pathogens, and 10 of them are more abundant in indoor environments. Previous studies also identified airborne pathogens in both indoor and outdoor environments [18,40]. Guo et al. detected 30 potential pathogens in the indoor air from 28 residences, 4 schools, and 2 office buildings [40]. Our previous study observed 23 pathogenic bacteria species in outdoor airborne particulates [18]. The above results show that pathogens accounted for a substantial part of the bacterial community, especially in indoor environments.

3.2. The health effects of pathogens and probiotics in indoor and outdoor environments

Given the wide existence of pathogens, we further identified the potential pathogens in our samples based on the FAPROTAX database and previous studies [41–48]. A total of 24 pathogenic species belonging to 18 genera were detected in indoor and outdoor environments (Table S5). The genus *Staphylococcus* comprised the most species of

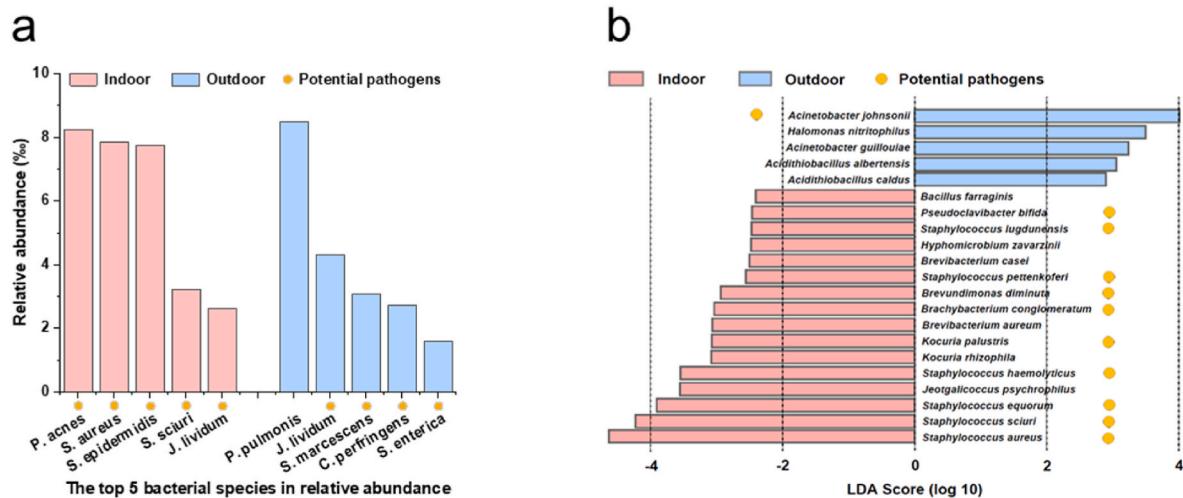


Fig. 2. The dominant species and feature species of the bacterial community in indoor and outdoor environments. (a) The top 5 species among all the classified species in relative abundance from the indoor and outdoor environments. (b) The linear discriminant analysis (LDA) scores of the feature species driving the difference of bacterial community in indoor and outdoor environments. Pink columns represent the species that were more abundant indoors while blue columns represent the species that were more abundant outdoors. The yellow cycles in the (a) and (d) mean the potential pathogens. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

potential pathogens. Some of the species held high relative abundances on average, such as *S. epidermidis* and *S. aureus* with a proportion of 4.66% and 4.25%, respectively (Table S5). *S. epidermidis* is known as an accidental pathogen, which rarely causes infection for healthy people but may endanger newborn babies and the immune-compromised cohort [47]. *S. aureus* is one of the most common pathogens causing various diseases, e.g., cellulitis [41], toxic-shock syndromes (TSS) [42], endocarditis [43], gastroenteritis [44], pneumonia [45], septicemias, and septic arthritis [46], etc. (Table S7) *Propionibacterium acnes* was another common pathogen in indoor and outdoor environments with an average relative abundance of 4.19% (Table S5). *P. acnes* was regarded as an opportunistic pathogen [48], contributing not only to acne vulgaris but also to endocarditis, dental infections, ocular infections, post-neurosurgical infections, etc. (Table S7) [28]. Overall, the pathogens detected in indoor and outdoor environments pose potential threats to the circulatory system, skeletal system, integumentary system, digestive system, and respiratory system (Fig. 3a).

On the other hand, the probiotics in indoor environments may bring benefits to occupants. Five probiotics, i.e., *Lactobacillus reuteri*, *Lactobacillus brevis*, *Lactobacillus salivarius*, *Lactobacillus delbrueckii*, and *Bacillus coagulans* were detected in our study (Fig. 3b; Tables S6) and 4 of which belong to the *Lactobacillus* genus which is one of the most widely used probiotics all over the world [49]. The observed *Lactobacillus* genus was conducive to the digestive system and immune system, helping in the prevention and/or treatment of diarrhea, caries, atopic dermatitis, etc. (Table S8) [50,51]. The other probiotics *Bacillus coagulans* can promote intestinal digestion and enhance the host immune system [52, 53]. To sum up, the observed probiotics mainly benefit the immune and digestive systems (Fig. 3b; Table S8).

We compared pathogens and probiotics between indoor and outdoor environments. The results showed that the overall relative abundance for all pathogens in indoor environments (34.12%) was twice that in outdoor environments (16.72%) (Fig. 3a; Table S5). The indoor/outdoor ratio of the overall relative abundance for probiotics was even higher, reaching ca. 8/1 (Fig. 3b; Table S6). The higher proportion in indoor environments for both pathogens and probiotics may be caused by the occupants, as most of the dominant pathogens and probiotics observed in the indoor environments were inhabitants of human bodies. For example, pathogens, e.g. *S. aureus*, *S. epidermidis*, and *P. acnes* are ubiquitously found on human skin and mucosal surfaces [28,47]. The probiotics, e.g. *L. reuteri* was found in the human gastrointestinal tract, urinary tract, and skin [50]. It is reasonable to assume that occupants play a critical role in shaping the distribution of indoor pathogens and probiotics.

3.3. Emission rates of pathogens and probiotics from occupants

The number of occupants was suggested as an important factor for the formation of bacterial communities [54,55]. In our results, the concentration of bacteria in indoor environments ranked as follows: office ($7.61 \times 10^5 \pm 1.86 \times 10^4$ copies·m⁻³ air), dormitory ($1.78 \times 10^5 \pm 4.25 \times 10^3$ copies·m⁻³ air) and residence ($3.87 \times 10^5 \pm 2.54 \times 10^4$ copies·m⁻³ air) (Table S4), in conformity with the rank of occupant density: office (0.35 person·m⁻²), dormitory (0.31 person·m⁻²) and residence (0.10 person·m⁻²) (Table S1). Chegini et al., also reported the concentration of indoor bacteria increased with increasing number of occupants [3]. Besides the overall concentration, the same pattern was also found in bacterial richness (observed species, Chao1, ACE) and diversity (Shannon, Simpson) which were highest in the office while lowest in the apartment (Fig. 4a and b). Barberán et al. also found that occupants had a strong influence on the composition of indoor bacteria [5].

To quantify the occupant contribution to indoor bacteria, we calculated the bacterial emission rates in occupied rooms (working place, bedroom, and lavatory) by the culture-dependent method. As a major pathogen observed in our study, *S. aureus* was selected as the indicator of

pathogens. For probiotics, *Lactobacillus* was chosen as the indicator because it comprises most kinds of probiotics. On average over all sampling rooms, the emission rates of total bacteria, *S. aureus*, and *Lactobacillus* were 23380, 1508, and 3994 CFU·person⁻¹·h⁻¹, respectively (Fig. S1). The average emission rate of total bacteria was twice that in a middle school (13620 CFU·person⁻¹·h⁻¹) [16]. Specifically, the emission rates of *S. aureus* and *Lactobacillus* were highest for the lavatory when occupied. The average concentrations for total bacteria, *S. aureus*, or *Lactobacillus* were all highest in the lavatory under occupied conditions as well (Fig. S2). The lavatory is a primary source of bacteria from the occupants' gut [56]. Some pathogens and probiotics, e.g., *S. aureus* and *Lactobacillus* which exist in the excrement were capable of dispersing into the air as bioaerosols [9,57]. Therefore, *S. aureus* and *Lactobacillus* have more chances to disperse into the lavatories than the working places and bedrooms. However, the emission rate for total bacteria was highest in the bedroom where the skin emission is dominant. This is not a surprise since the skin harbors millions of bacteria and functions as a major source for indoor bacteria [1].

The gender effects were also investigated for the lavatory and bedroom where there are separate spaces for males or females. The average emission rates of total bacteria and, notably, *Lactobacillus* in female lavatories were 3.8 and 9.3 times higher than those in male lavatories, respectively (Fig. 4c). These results are consistent with Barberán et al.'s study showing *Lactobacillus* in homes was more abundant when females were present likely since many species of *Lactobacillus* exist vaginas [5]. It is intriguing to see that women appeared to be an important source of probiotics. For bedrooms, the emission rate of total bacteria, *S. aureus*, and *Lactobacillus* were slightly higher in male rooms than those in female rooms (Fig. 4d). People usually dress less and show more skins in bedrooms. Due to the larger body sizes, males shed more bacteria than women [58]. The previous study was also found that skin-associated bacteria were more abundant in homes with more males [5].

4. Conclusion

With the aid of the culture-independent method, the differences between the bacterial distributions in indoor and outdoor environments were manifested in terms of concentration, relative abundance, and feature species. The health impacts (negative or positive) of the identified bacteria were presented based on the FAPROTAX database. The observed pathogens pose potential threats to the circulatory system, skeletal system, integumentary system, digestive system, and respiratory system while the observed probiotics are potentially beneficial to the immune and digestive systems.

Occupants played a profound role in shaping the bacterial distribution in indoor environments, acting as a major source for both pathogens and probiotics. The contributions of occupants were quantified by emission rates via the culture-dependent method and they varied in different indoor spaces (working place, bedroom, and lavatory) with different genders (male and female). The average emission rates in lavatories were highest for *S. aureus* (a representative for pathogens) and *Lactobacillus* (a representative for probiotics) while that for total bacteria was highest in bedrooms. *S. aureus* is commonly found in the gut while *Lactobacillus* exists in vaginas, explaining the highest emission rates observed in lavatories. It is worth noting that the emission rate of *Lactobacillus* in the female lavatories was much higher than that in the male lavatories, indicating females are potentially an important source for probiotics in indoor environments.

It should be pointed out that the effects of dispersion, deposition, biological decay, and metabolisms of bacteria were not included in our study. Future studies that employ bacterial tracers to probe the transmission processes of pathogens and probiotics, and predict the bacterial effects on human health more accurately are suggested.

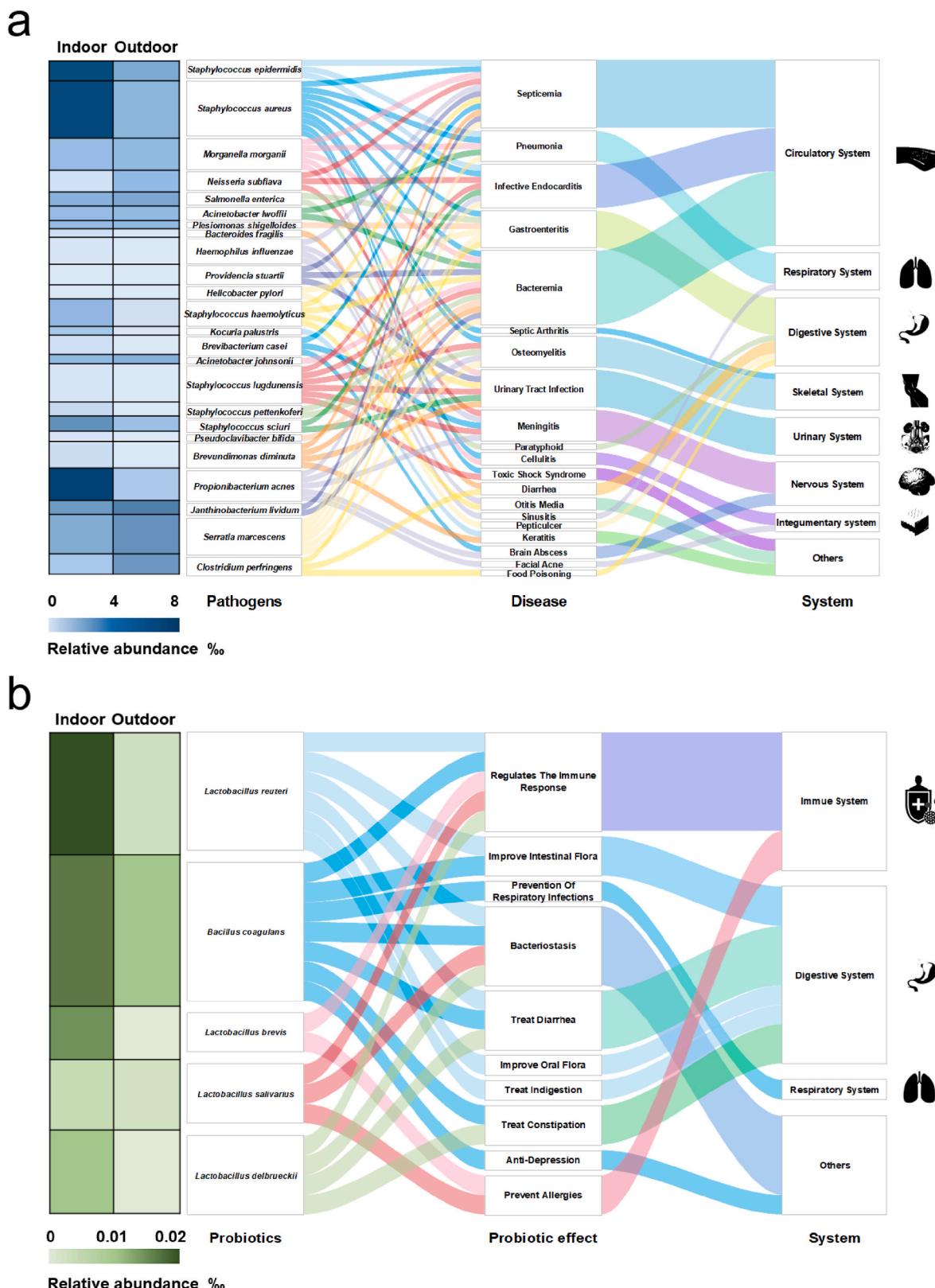


Fig. 3. The health effect of pathogens and probiotics in indoor and outdoor environments. (a) The relative abundance and potential of health risks of pathogens. (b) The relative abundance and health benefits of probiotics. On the left side of the heatmap, the squares with different degrees of blue and green colors represent the different relative abundance of pathogens and probiotics, respectively. The alluvial diagrams suggest the relationships of pathogens or probiotics, possible diseases or probiotics effects, and human body systems. Different symbols on the right side represent different human body systems. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

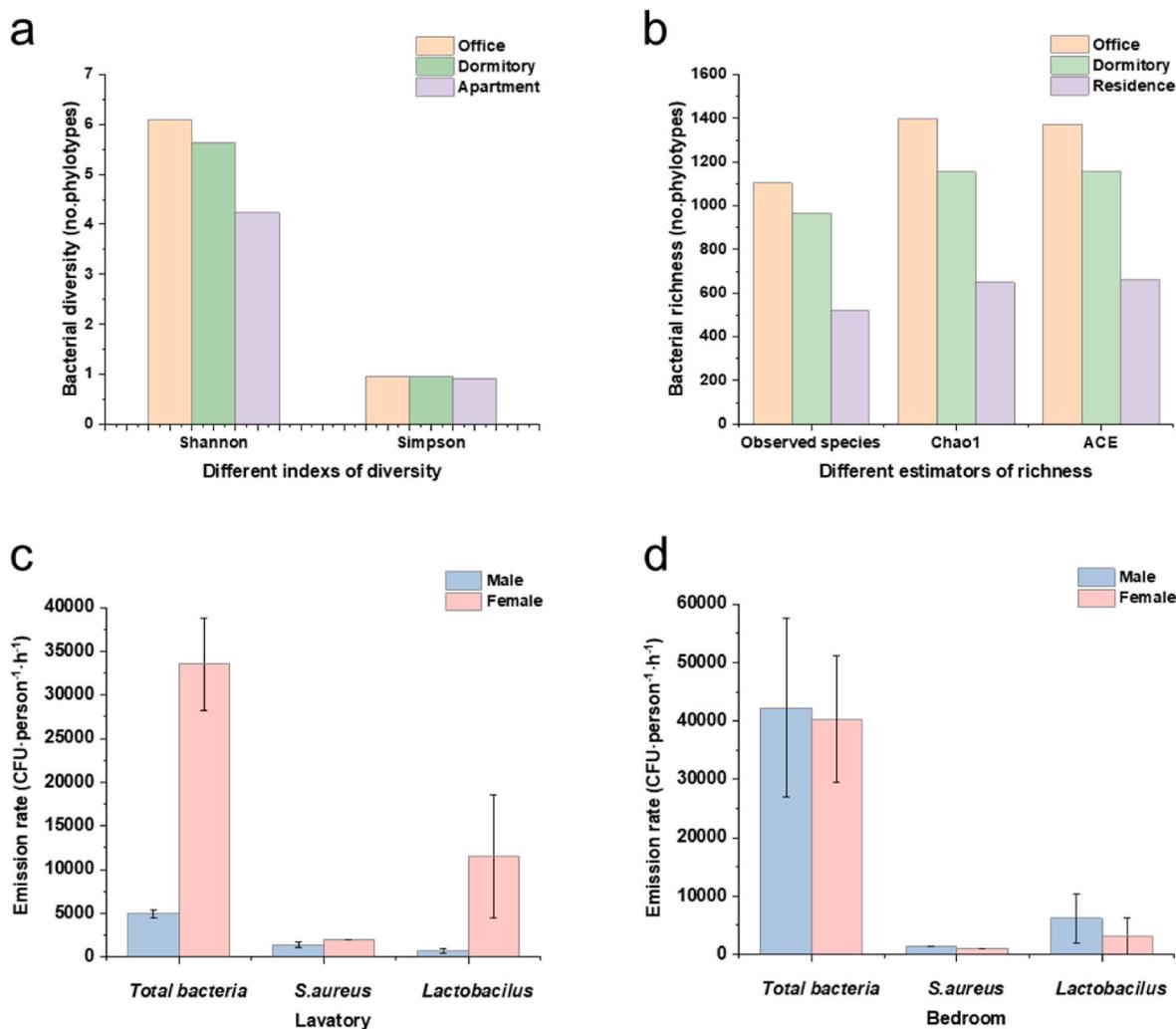


Fig. 4. The community characters and emission rate of bacteria in indoor environments. (a) Different indicators (observed species, Chao1, and ACE) of richness for the bacterial community. (b) Different indicators (Shannon and Simpson) of diversity for the bacterial community. The average emission rate of total bacteria, *S. aureus*, and *Lactobacillus* in lavatories (c) and bedrooms (d) with different genders. Working places were not included in the comparison of genders since there are no separate spaces for females and males. Blue colors represent the males and pink colors represent the females. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

CRediT authorship contribution statement

Meng Liu: Writing – review & editing, Supervision, Conceptualization. **Zifeng Gan:** Data curation, Methodology, Software. **Bingyang Shen:** Visualization, Investigation. **Lumeng Liu:** Supervision, Writing – review & editing. **Wenmao Zeng:** Investigation. **Qisheng Li:** Investigation. **Huan Liu:** Writing – original draft, Validation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.buildenv.2022.108863>.

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