

Understanding effects of ventilation on airborne microorganisms in build environments: A perspective

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Abstract. The indoor air quality is associated with occupant productivity and a host of chronic health problems, including allergies, asthma, and depression. Ventilation is one of the solutions to improve air quality. Qualitative and quantitative characteristics of different ventilation systems, i.e., natural, mechanical and hybrid systems, might have an influence on several aspects of indoor environmental quality. As potential indoor pollutants, there are a great variety of components such as chemical substances and microbes, but our knowledge about the relationship between ventilation and microbes inhabiting the built environment is limited, including SARS-CoV-2. This limitation may partly be caused by the facts that i) methods, especially sampling of low concentration microbes from the air, for investigating indoor microbial community have not yet been established, ii) microbes in the built environment are greatly influenced by the surrounding environment and human lifestyle and behavior, and iii) different ventilation methods also affect the microbial community. The purpose of this study is to summarize the importance of airborne microorganisms in the built environment, focus on very different built environments with natural and mechanical ventilation, respectively, from a microbiological view, and attempt to find the characteristics of microbial communities in each environment. As a result, the possibilities and limitations of the current ventilation systems are highlighted, as well as tools and methods useful for analyzing airborne microbial communities, with preliminary results from our new-generation sequencer.

Keywords. Ventilation, public health, microbiome, airborne microorganism, bioaerosol.

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1. Introduction

Humans spend most of their time in indoor environments, and in recent years, infections and allergic diseases caused by inhalation of indoor bioaerosols have become a problem. Deteriorated indoor air quality (IAQ) presents one of the most critical health risk factors in living and working environments, where inadequate air quality parameters (i.e., qualitative, and quantitative) might affect the health, comfort, and productivity of building occupants [1,2,3]. Several research works over the world found the strongest association of health issues with IAQ among many quality factors such as thermal comfort, noise and acoustics, and daylight in indoor environment [4]. The engineering measures and environmental health activities have to be directed toward attaining the optimal IAQ, which is an essential precondition for protecting users' health. That results in saved healthy life years

[5] and decreased number of deaths, and significant economic savings [2,3]. According to the report by WHO in 2016 [5], 4.3 million people's death per year globally can be decreased if we prevent their exposure to household air pollution. Recent cost-benefit analysis has shown that costs of the poor indoor environment for the employer, the building owner, and society are often considerably higher than the cost of the energy used in the same building. Ventilation plays one of the essential roles to solve this problem. The general purpose of ventilation in buildings is to provide healthy air for breathing by both diluting the pollutants originating in the building and removing the pollutants from it [6]. Regarding selected pollutants guideline, WHO published another guideline in 2010 that considered the following substances: benzene, carbon monoxide, formaldehyde, naphthalene, nitrogen dioxide, polycyclic aromatic hydrocarbons (especially benzo[a]pyrene), radon, trichloroethylene, and

tetrachloroethylene [1]. Each pollutant was described on general description, indoor sources, pathways of exposure, indoor concentrations, indoor-outdoor relationship, kinetics and metabolism, health effects, health risk evaluation. However, there is a lack of ventilation studies, especially in the area of synergistic interactions of chemical pollutants and microbes. Therefore, we summarized the importance of airborne microorganisms in the built environment, focusing different built environments with natural and mechanical ventilation, respectively.

2. Airborne microorganisms in built environments

Microorganisms are tiny organisms (ranging from 0.002 to 100 μm in size), often single celled and invisible to the naked eye. In this perspective, when we talk about airborne microorganisms, it contains the conditions such as airborne ($\leq 5 \mu\text{m}$), droplet, and falling.

2.1 importance of airborne microorganisms

Biological agents affect building materials and the built environment as well as human health. They are widely heterogeneous, ranging from pollen and spores of plants (mainly from outdoors) to bacteria, bacterial endotoxins, archaea, fungi, algae, and some protozoa emitted outdoor and indoors. Some of the airborne algae, moulds, lichens, and mosses that grow on walls can cause the soiling of building surfaces [7] and sometimes even cause concrete degradation [8].

The air inhaled by a human typically contains 10^6 airborne microorganisms/day [9]. Among the one to ten million species of microorganisms on the earth, scientists estimate that less than one percent cause disease [10]. The rest may contribute to the ecosystem, including humans. Their mechanisms are not yet clearly understood, however, recently diversity of microbial exposure has been associated with a reduced risk of asthma, atopic dermatitis and allergic sensitization in population studies [11,12]. For example, the human gut contains microbes that are essential for digestion and to produce vitamins, antimicrobials, and neurotransmitters [13,14]. On the other hand, some fungi such as *Aspergillus versicolor*, *A. alternata*, *A. niger*, and *Penicillium* positively related to allergic diseases and asthma [15]. Also, other airborne pathogenic bacteria like *Legionella pneumophila*, *Mycobacterium*, *Micrococcus luteus*, *Staphylococcus aureus*, and the component of the exterior cell-wall of Gram-negative bacteria (endotoxins) could perform infectivity and allergenicity [16]. Influenza-infected patients generate < 20 influenza virus RNA particles per minute just by breathing [17], and it is known that each sneeze releases about 200 million virus particles [18]. A single sneeze produces about 4,600 – 40,000 microbe-filled particles at a velocity of 360 km/h, and a single cough generates about 3,000

droplets [19,20]. Just by taking these things into consideration, the quality and quantities of the airborne microorganisms in a built environment are closely related to human health both in a positive and negative way.

2.2 source of airborne microorganisms

Like our bodies, the buildings we live in are filled with microbes. Occupants, water, building materials, and ventilation are important sources of microbes in the built environment [21,22,23]. When a resident moves to a new home, it may take only a few days for his or her unique microbiome to rebuild in the new location [24]. The number of occupants (including pets and plants), gender and room specifications for activity areas (bathrooms, kitchens, etc.) affect the bacterial communities [21,25]. Scientists also found that water significantly impacts building damage and microbial growth. The number of culturable fungi was about 20 times higher in homes with moderate/heavily water damage than in homes with mildly water damage, and mould, endotoxins, and fungal glucans were detected in the environment at concentrations that could cause health effects [26]. The impact of flooding on the microbiome continued even after the relative humidity had returned to baseline and residents had removed flood-damaged items and renovated damaged rooms [27]. Ventilation is also known as one of the major sources of the indoor microbiome. Within offices, the source of ventilation air had the greatest effect on bacterial community structure [28], and at high outdoor air ventilation rates, the bacterial community in the indoor air tended to be closer to the bacterial community in the outdoor air [29,30]. Another study indicates that indoor fungal communities were originally come from outdoors [31].

3. The impact of ventilation on indoor airborne microbial communities and their concentration

3.1 microbes in a completely mechanically ventilated built environment

Theoretical analysis of housing types suggests that reduced ventilation has potential health effects, including the transmission of infectious diseases. Studies have also shown that building design affects the indoor microbial community, that mechanically ventilated buildings have lower microbial diversity than naturally ventilated buildings, and that the microbes present in mechanically ventilated buildings have a high percentage of taxa closely related to potential pathogens and a very low presence of environmental taxa [32,33]. We chose the internal space station (ISS) as a case study of an airtight built environment because there is no air outside the ISS, and outdoor sources are negligible. Mechanical ventilation is controlled to remove the target particle size range inside the ISS: the ISS filters

media, called a bacteria filter element (BFE), has a 99.97% efficiency rating in removing particles of 0.3 µm diameter. It means this filter removes bacteria and larger particles from the air before flowing into the ISS air reclamation system for reuse [34]. Of six references on microbial community analysis of the ISS, two were using swabs [35,36], one was using the contents of a vacuum cleaner bag (ISS-debris in the paper) [37], and the remaining three were using aerosol filters or HEPA filters [34,38,39]. As a result, two fungal genera, *Aspergillus* and *Penicillium*, were commonly found in all studies, both by culture methods and by using culture-independent methods with a new generation sequencer (NGS). While, in the case of bacteria, no genus was found to be common to all the study by culture method, and two genera, *Staphylococcus* and *Streptococcus*, were found to be common by NGS analysis (Tab. 1). All those four genera are ubiquitous microorganisms in built environments. *Aspergillus-Penicillium* type spores are known to be the most prevalent in the indoor air of residential properties [40]. Regarding the bacteria, these two genera have been detected as originating from humans, supporting previous research [15,16,17]. They are almost ubiquitously found in the nasal cavity for *Staphylococcus* and in the oral cavity for *Streptococcus*, suggesting that these bacteria were released from humans, especially through nasal and oral respiration. Another potentially major source of airborne microorganisms, faecal-derived species such as *Escherichia*, have not appeared as common genera. It is probably because most of these bacteria do not have desiccation tolerance, unlike *Staphylococcus* and *Streptococcus*.

Tab. 1- Commonly found fungal and bacterial genera in selected studies. Only genus-level in taxonomy data were compared.

Fungi		Bacteria	
Culture	Sequencing	Culture	Sequencing
<i>Aspergillus</i>	<i>Aspergillus</i>	- ^a	<i>Staphylococcus</i>
<i>Penicillium</i>	<i>Penicillium</i>		<i>Streptococcus</i>

a. No common bacterial genus was found in all the studies.

3.2 microbes in a highly naturally ventilated built environment

Recently, a few studies suggest that opening windows itself does not have a significant impact on the indoor airborne microbial community. This may be due to the fact that the studies were performed at homes without envelope airtightness. While it is well known that opening windows and ventilating a room primarily through outside air results in higher bacterial diversity than those with the windows are closed, and the room is ventilated using an HVAC system [28]. Also, in buildings with a large amount of outdoor air, such as traditional houses and houses in a rural area (Fig. 1), the bacterial community in the indoor air tends to be closer to the bacterial community in the outdoor air [21,41,42]. As

mentioned above, the bacterial genus of *Staphylococcus* and *Streptococcus*, and the fungal genus of *Aspergillus* and *Penicillium* are common and abundant in the built environment with mechanically ventilated. It is also shown that indoor air contains significantly more potentially harmful bacteria than outdoor air [43]. While in the built environment with naturally ventilated, *Microcystis*, *Prochlorococcus*, and *Methylocella* are common bacterial genus [20]. Other studies have shown that the bacterial genera *Rhodanobacter*, *Gemmatimonas*, and *Reyranelia* are more common in rural buildings [44], and *Hymenobacter* and *Solirubrobacter* are the indicator bacteria in rural areas [45].

Studies have shown associations between increased ventilation rates and improved health outcomes, including reduced incidence of influenza and asthma, and allergy symptoms [46,47]. In outdoors, the fine particles that we cannot see are almost always quickly dispersed, and when viruses released into the air are rapidly diluted, carried by wind currents, and spread over seemingly infinite space. Sunlight also inactivates the virus [48]. Exposure to certain microbes is especially important at an early age when the immune system is still developing. For example, it is known that children living in homes with pets such as dogs and cats are less likely to develop asthma and allergies than those without pets [12,49]. Unlike pathogens, there is little evidence of which microorganisms are good for health. It could be a combination of several species rather than a specific substance or microbes. In the future, if the relationship between natural ventilation and health is elucidated, we may be able to design a human- and eco-friendly ventilation system.



Fig. 1- Traditional Japanese house with a thatched roof. In summer, windows and partitions are opened, and in winter, windows are closed with the use of a wood stove.

3.3 impact of ventilation rate and types on indoor airborne microbial concentrations

There are controversial reports regarding the effect of ventilation on the airborne microorganisms. Some studies show that the effect of outdoor air ventilation rate on the concentration of bacteria and fungi in indoor air is not significant [43, 50]. On the other hand, previous studies have shown that hospital areas with natural ventilation have bioaerosol concentrations as 10 times higher than those with conventional mechanical ventilation systems [51].

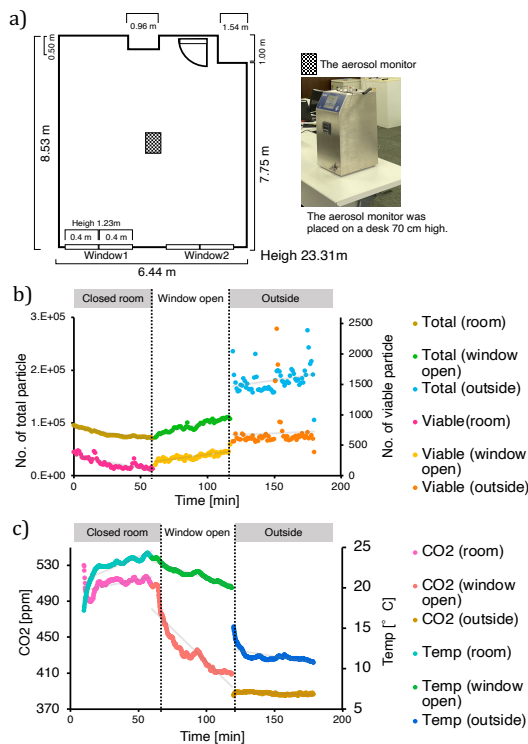


Fig. 2- a) Numbers of total particles and viable microorganisms was monitored using a real-time viable particle counter (BioTrak, TSI Inc., Shoreview, MN, USA) in a room. The room size is approximately 1,280 m³ and there were 5 people in it. The counter was placed in the center of the room, and on a desk 70 cm high. There were two windows and one door. The area of the open window is 0.49 m². b) For the first 1 hour, windows and the door were closed and mechanically ventilated; for the next 1 hour, window1 and the door were opened. For the last 1 hour, the counter was placed outside and monitored. c) Temperature and CO₂ concentrations were measured using sensors RTR-576 (T&D Corp., Nagano, Japan).

Our unpublished observation also showed the ventilation from a window had an impact not only on the number of total particulate matter but also on the number of viable microorganisms. During natural ventilation with open windows for one hour, when the number of particles in the outdoor air was higher than that in the indoor air, the number of particles in the indoor air increased (Fig. 2a, b). While, both CO₂ concentration and air temperature were lower outside, and both became lower inside when

windows were opened (Fig. 2c). These results indicate that particles from the outdoor air entered the room and may influence the indoor microbial concentration as well as the community composition.

4. Useful tools/methods for airborne microbial community analysis

Culture-independent methods have been developed such as fluorescent microscopy, quantitative polymerase chain reaction (qPCR), digital PCR, and metagenomic approaches targeting small subunit ribosomal RNA gene with new generation sequencer. A growing number of studies have been conducted with these new techniques, in which the concentration of bioaerosol is up to 1,000 times higher than those with conventional culture methods [52]. Furthermore, culturable bacteria account for only 1-20% of the total bacterial diversity [53]. Aerosol sampling methods are quite complicated, including impaction, impingement, filtration, gravity sampling, electrostatic precipitation, cyclone methods, thermal precipitator, and condensation technique [54]. The sampling method used for studying airborne microorganisms is important because the concentration of biomass in samples is generally low, and the yield obtained depends on the collection device, sample matrix (dry/liquid, type of medium, filter, and buffer with/without DNA/RNA later, etc.), sampling time, and speed. Although there are standard operating procedures for marine and soil environmental samples, the development of standardized protocols for bioaerosol research is still in its beginning stages. In addition, it is worth noting that the efficiency of the DNA extraction method and the purity of the obtained DNA need to be improved. The following section shows our unpublished example of the effect of different DNA extraction methods on the microbial community structure.

4.1 air sampling

Air samples were collected on the roof of the three-story building of the Faculty of Science, the University of Toyama (36°41'54"N, 137°11'13"E, 23 m above mean sea level, AMSL) in August 2017 using a Teflon membrane filter (PTFE membrane, 46.2 mm

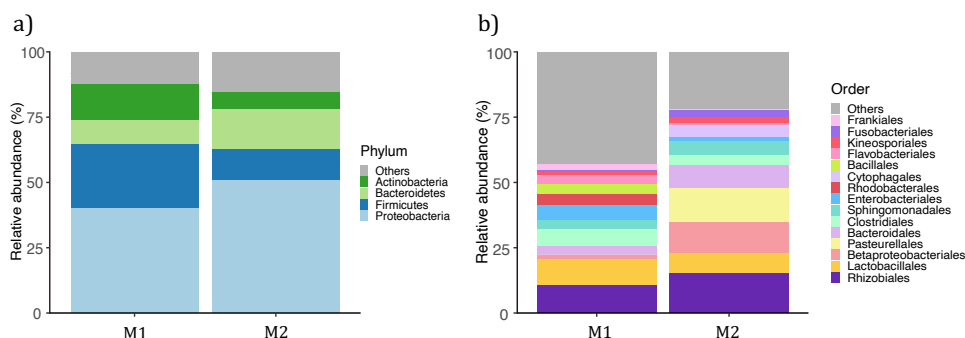


Fig. 3- Differences in DNA extraction methods and bacterial community. At the phylum level, the bacterial community was almost the same in both M1 and M2 extraction methods (a), while there was a difference in the bacterial community at the order level (b).

diameter, 2 μm pore size, GVS Japan KK, Tokyo, Japan) and slit jet air sampler (MCAS-SJ, Murata Keisokuki Service Co., Ltd., Kanagawa, Japan) at a flow rate of 30 L/min for 23 h. A total volume of 42 m³ of air was collected. After sampling, the filter was immediately carried to the laboratory and cut in half with sterile scissors under a laminar flow cabinet.

4.2 DNA extraction

Method 1 (M1): a half-cut filter was directly placed in a bead tube of a DNeasy PowerBiofilm Kit (QIAGEN, Germantown, MD, USA) under a laminar flow cabinet, and DNA was extracted as described previously [55].

Method 2 (M2): the other part of the filter was directly placed in a bead tube of a FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) under a laminar flow cabinet, and DNA was extracted according to the manufacturer's protocol.

Each DNA sample was quantified using a DS-11FX+ Spectro/Fluorometer (DeNovix, Wilmington, USA) and a QuantiFluor™ dsDNA System (Promega, Madison, USA), and stored at -30°C until sequencing.

4.3 sequence and data analysis

PCR conditions, sequencing library constructions, sequencing using MiSeq (Illumina, San Diego, CA, USA) and a Miseq reagent kit V3 600 cycles (Illumina), taxonomic assignment with DADA2 v.1.14.1 [56] on SSU Ref tree of SILVA release v.132, data analysis with phyloseq v.1.38.0 [57] and vegan v.2.5.7 were performed as described in Yarimizu et al., 2021 [58].

4.4 DNA extraction methods result in different microbial community and diversity

In our study, the same filter sample was cut in half and DNA was extracted using different methods (M1 and M2), resulting in the different microbial community: the microbial community was similar at the phylum and class levels (Fig. 3a), but the one at the order level in taxonomy was different (Fig. 3b). Furthermore, the number of operational taxonomic units (OTUs) detected and alpha-diversity using the Shannon index, which is an index of microbial diversity based on the species richness and its evenness, differed depending on the extraction method (Fig. 4a, b). Currently, molecular biological and immunological detection methods and culture methods are mainly used for the detection of

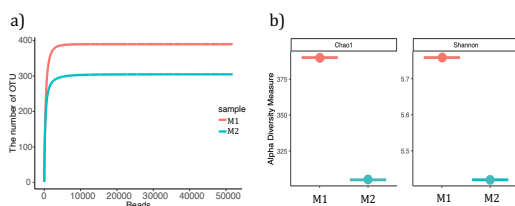


Fig. 4- Differences in the diversity of microbial community structure by DNA extraction method. Both at the OTU level (a) and in Shannon diversity (b), extraction method M1 has a higher microbial diversity than that of M2.

microorganisms, including pathogens [59]. Like other physicochemical metrics such as CO₂, Volatile Organic Compounds (VOC), Particulate Matter (PM), radon, temperature, and relative humidity, the ability to monitor the species and quantity of airborne microorganisms, including pathogens in real-time would facilitate rapid assessment of potential airborne microbial contamination and help improve the management and maintenance of indoor air quality.

5. Conclusion

This study systematically reviewed recent papers to elucidate the impact of mechanical/natural ventilation on the type and concentration of indoor airborne microorganisms. The fact that a mechanically ventilated-only built environment does not differ significantly from the microbes that appear in a typical built environment suggests mechanical ventilation works well to maintain airborne microorganisms in some parts. Recently, energy-efficient buildings with high building airtightness and minimal ventilation are increasingly being used as eco-friendly homes. Regarding the effect of ventilation on the airborne microbial community, there are controversial reports to maintain the microbial diversity in such high airtightness-built environments. Through our reviewing the conflicting reports, we measured the concentration of indoor airborne microorganisms, resulting in that natural ventilation affects indoor microbial concentrations. In addition, the composition of the microbial species varied greatly depending on the DNA extraction method, indicating that methodological standardization is a first essential step for indoor airborne microbiological analysis. Qualitative and quantitative monitoring of airborne microorganisms in the built environment is important from the perspective of public health, and the first priority should be to establish systems that allow constant monitoring of microbial concentrations in airborne particle counts like physicochemical factors such as CO₂, temperature, relative humidity, daylight, radon, etc., and periodically (e.g., seasonally or annually) analyse the dynamics of airborne microbial communities including pathogens. The built environment, which is said to be good for human health, may not be a closed system. In order to understand the fluctuations of airborne microbial communities in the built environment, comprehensive research is needed on the dynamics of suspended particles, human behaviour, living space, neighbourhoods, and architectural design as a "meta-community" [60] that considers the entire community as an ecosystem. We are currently working on expanding the local and global bioaerosol research network through CHOBE (Center for Holobiome and Built Environment) [61] and BioskyNet [62]. In the future, further development of global (global + local) aerosol research and the definition of a "healthy" indoor and outdoor microbiome will open the window to a more comfortable and healthier living environment.

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Data access statement

The datasets generated during and/or analysed during the current study are available in the DDBJ repository, under the accession number DRA012566.