

Dear Editor and Reviewers,

We thank the referees for their careful reading and constructive comments, which have allowed us to make further enhancements and significantly raised the quality of the manuscript. Our responses to the comments from the three referees are provided below. The reviewers' comments are shown in blue, while our responses are in black. All page and line numbers mentioned in the response refer to the revised version of the manuscript. Revisions are marked in blue in the revised manuscript. The revised manuscript and supplement (see marked copy) are provided below the response.

Sincerely,

HU Wei (on behalf of co-authors)

Response to Referee 1:

This work from Jin et al., titled with “Exometabolomic exploration of culturable airborne microorganisms from an urban atmosphere” has investigated the metabolic activity of airborne culturable microorganisms at the molecule level. Distinct metabolic profiles were observed for the different bacterial strains and the fungal strains. Some suggestions are as below:

R1 Comment 1: It is interesting to reveal the different metabolic profile of microorganisms isolated from the ambient air. How about the abundance of these unique “produced” products in the ambient air?

Response: Thank you for your valuable comments. It is feasible that atmospheric microorganisms could contribute to biogeochemical processes through metabolic activities, including carbon fixation or degradation of carbon compounds (e.g., pollutants). In this study, analysis of atmospheric microbial exometabolites revealed many molecules that existed in ambient aerosols, such as myristic acid ($C_{14}H_{28}O_2$), stearic acid ($C_{18}H_{36}O_2$), palmitic acid ($C_{16}H_{32}O_2$), linoleic acid ($C_{18}H_{32}O_2$), linolenic acids ($C_{18}H_{30}O_2$) and other lipid-like molecules.

We agree that the understanding on the abundance of these unique exometabolites produced by airborne microorganisms in ambient air is an important aspect of their ecological role. However, our current study primarily focused on characterizing the metabolic profiles of the isolated microorganisms under controlled conditions to assess their potential impacts on atmospheric chemistry and biogeochemical processes. However, because of technical limitations, the metabolite analysis methods used in the study can perform qualitative and semi-quantitative analyses but cannot provide accurate quantitative results. In future metabolomics analyses of ambient air samples, we need to explore the abundance of microbial exometabolites by using high-resolution quantitative analytical methods. Our current findings can serve as a database of atmospheric microbial exometabolites to better assess their ecological significance in the atmosphere.

R1 Comment 2: The introduction is somewhat lengthy; it is recommended to condense and refine it appropriately.

Response: Thank you for your comments. We have condensed and reorganized the introduction section, which is not shown here because of its length but is marked in blue in the revised manuscript. Briefly, we streamlined the discussion on the oxidative degradation of organic matter by atmospheric microorganisms, focusing on the most pertinent findings. Specifically, we removed the section discussing the link between atmospheric microorganisms and inorganic oxidants, as it was less central to the study's scope. Additionally, we reorganized the section on the importance of metabolomic studies, placing greater emphasis on findings directly relevant to atmospheric microbial metabolites. These revisions aim to enhance clarity and ensure the introduction is more concise while providing a solid foundation for the research presented. The revisions are marked blue in the revised manuscript in Page 2, Line 52 – 72 and Line 83 – 96.

R1 Comment 3: Lines 150-160: why the culture process were repeated for three cycles? What is the rationale behind this? Bacteria were culture at 37 °C and fungi were cultured at 28 °C. Why these two temperatures were selected? will the culture temperature affect the exometabolome?

Response: In the isolation experiments, we checked the stability of each strain by repeating the culture process through multiple cycles, ensuring consistency and reproducibility of the organisms. Typically, environmental microbial researchers conduct microbial isolation by culturing at least three cycles in most studies (Dunbar et al., 2015; Timm et al., 2020; Yan et al., 2021). In our present study, we achieved stable and robust growth of the isolated microbial strains after performing three or four streaking cycles. However, when encountering poor growth or inconsistent single colonies during strain isolation, we can conduct additional repetitions to ensure the acquisition of pure single colonies.

In the revision, we added more details on the microbial cultivation and isolation processes in Line 134 – 138, Page 5.

“From each plate, all phenotypically distinct colonies were picked up and smeared onto fresh media for isolation. Single colonies were picked and re-streaked from each plate at least three times to isolate individual strains (Timm et al., 2020). Multiple streaking isolations are essential to obtain stable and pure strains by gradually diluting the microbial population and allowing for the isolation of individual colonies (Yan et al., 2021; Dunbar et al., 2015).”

In this part of the method section, we reference the following literatures:

Dunbar, D., Shade, D., Correa, A., Farina, J., Terry, K., Broomell, H., Graves, L., Chudoff, D., Schoff, C., and Cross, T.: An Optimized Enrichment Technique for the Isolation of *Arthrobacter* Bacteriophage Species from Soil Sample Isolates, Journal of Visualized Experiments, e52781, <https://doi.org/10.3791/52781>, 2015.

Timm, C. M., Loomis, K., Stone, W., Mehoke, T., Brensinger, B., Pellicore, M., Staniczenko, P. P. A., Charles, C., Nayak, S., and Karig, D. K.: Isolation and characterization of diverse microbial representatives from the human skin microbiome, Microbiome, 8, 58, <https://doi.org/10.1186/s40168-020-00831-y>, 2020.

Yan, D., Zhang, T., Bai, J.-L., Su, J., Zhao, L.-L., Wang, H., Fang, X.-M., Zhang, Y.-Q., Liu, H.-Y., and Yu, L.-Y.: Isolation, Characterization, and Antimicrobial Activity of Bacterial and Fungal Representatives Associated With Particulate Matter During Haze and Non-haze Days, Frontiers in Microbiology, 12, 793037, <https://doi.org/10.3389/fmicb.2021.793037>, 2021.

The temperatures used for culturing bacteria and fungi are typically chosen based on their optimum growth condition and are often detailed in the sections of materials and methods in literatures (Wang et al., 2023; Zhan et al., 2024; Liu et al., 2024; Yang et al., 2022; Yang et al., 2021).

The optimal temperature for culturing atmospheric bacteria depends largely on the specific species or strains involved. While temperatures between 30°C and 37°C are commonly used for a wide range of bacterial cultures, 37°C is frequently employed, particularly for species that are adapted to environments influenced by human activities. This temperature reflects conditions where human-associated microbial communities

thrive. In studies focused on airborne bacterial cultures, 37°C is often the preferred incubation temperature, as it is widely used in the isolation and count of bacteria from various environments, including caves, hospitals, schools, and sewage treatment plants (Tomazin et al., 2024; Jeong et al., 2022; Zhang et al., 2023).

On the other hand, many fungi, such as those found in soils or on decaying organic matter, prefer environments with moderate temperatures, typically ranging from 20°C to 30°C. Specifically, 28°C is often used in fungal cultures because it is slightly higher than room temperature and reflects the conditions many fungi encounter in their natural environments, supporting robust growth and replication for a wide range of fungal species (Riccardi et al., 2021; Wang et al., 2023).

To some extent, different temperatures affect the growth rate, metabolic activity, and overall behavior of microorganisms. In our study, we specifically focused on assessing the potential metabolic capacity of atmospheric microorganisms under optimal conditions. To achieve this, we carefully selected suitable temperatures to ensure we obtained the desired results.

In this part of the method section, we reference the following literatures:

Liu, Y., Deng, G., Liu, H., Chen, P., Pan, Y., Chen, L., Chen, H., and Zhang, G.: Seasonal variations of airborne microbial diversity in waste transfer stations and preventive effect on *Streptococcus pneumoniae* induced pulmonary inflammation, Science of The Total Environment, 912, <https://doi.org/10.1016/j.scitotenv.2023.168888>, 2024.

Wang, S., Qian, H., Sun, Z., Cao, G., Ding, P., and Zheng, X.: Comparison of airborne bacteria and fungi in different built environments in selected cities in five climate zones of China, Science of The Total Environment, 860, 160445, <https://doi.org/10.1016/j.scitotenv.2022.160445>, 2023.

Yang, L., Shen, Z., Wei, J., Wang, X., Xu, H., Sun, J., Wang, Q., and Cao, J.: Size distribution, community composition, and influencing factors of bioaerosols on haze and non-haze days in a megacity in Northwest China, Science of The Total Environment, 838, 155969, <https://doi.org/10.1016/j.scitotenv.2022.155969>, 2022.

Yang, L., Shen, Z., Wang, D., Wei, J., Wang, X., Sun, J., Xu, H., and Cao, J.: Diurnal Variations of Size-Resolved Bioaerosols During Autumn and Winter Over a Semi-Arid Megacity in Northwest China,

Geohealth, 5, e2021GH000411, <https://doi.org/10.1029/2021GH000411>, 2021.

Zhan, J., Xu, S., Zhu, Y., Han, Y., Li, L., Liu, J., and Guo, X.: Potential pathogenic microorganisms in rural wastewater treatment process: Succession characteristics, concentration variation, source exploration, and risk assessment, *Water Research*, 254, <https://doi.org/10.1016/j.watres.2024.121359>, 2024.

R1 Comment 4: Lines 223: what does this word “resistant” mean here?

Response: Thank you for your comments. The word “resistant” means peaks that appear in both the initial medium and the final culture solution. We mentioned the definition in Line 234, Page 8. These molecules are not entirely consumed by the microorganisms, although may be partially produced but cannot be distinguished as exometabolites. Similar terminology “resistant” is also used in previous literatures (Chen et al., 2023; Yu et al., 2019; Mitra et al., 2013).

Chen, H., Uzun, H., Tolić, N., Chu, R., Karanfil, T., and Chow, A. T.: Molecular Transformation of Dissolved Organic Matter during the Processes of Wildfire, Alum Coagulation, and Disinfection Using ESI(–) and ESI(+) FT-ICR MS, *ACS ES&T Water*, 3, 2571-2580, <https://doi.org/10.1021/acsestwater.3c00135>, 2023.

Mitra, S., Wozniak, A. S., Miller, R., Hatcher, P. G., Buonassissi, C., and Brown, M.: Multiproxy probing of rainwater dissolved organic matter (DOM) composition in coastal storms as a function of trajectory, *Marine Chemistry*, 154, 67-76, <https://doi.org/10.1016/j.marchem.2013.05.013>, 2013.

Yu, Z., Liu, X., Chen, C., Liao, H., Chen, Z., and Zhou, S.: Molecular insights into the transformation of dissolved organic matter during hyperthermophilic composting using ESI FT-ICR MS, *Bioresource Technology*, 292, 122007, <https://doi.org/10.1016/j.biortech.2019.122007>, 2019.

R1 Comment 5: Figure 2 panel b: it would be better to label each column. Otherwise, it is not clear which bar is the consumed, resistant, and produced organic matter.

Response: We have modified Figure 2 panel b by labeling the abbreviations of Consumed, Resistant, and Produced as C, R, and P below each column and adding a legend. All abbreviations are explained in the figure caption.

“Figure 2. Changes in molecular compositions of culture media during the metabolic processes of six typical atmospheric culturable bacteria. (a) Van Krevelen diagrams illustrate changes in different categories of organics in the media after incubation. The top three Van Krevelen diagrams represent the chemical composition of Gram-positive bacteria, the bottom three Van Krevelen diagrams correspond to Gram-negative bacteria. (b) The stacked bar charts indicate the formula numbers of different categories of consumed (labeled as C), resistant (R), and produced (P) organic matter.”

R1 Comment 6: Figure 4 pane a: I assume that the two pie charts represent the distribution of number concentration and intensity concentration, respectively. if yes, it might be better to use different color scales for these two charts to differentiate them. Similar suggestion for figure 5a.

Response: Thank you for your comments. The two pie charts in Figure 4a and Figure 6a (previously referred to as Figure 5a in the original manuscript) represent the number and intensity of molecules composed of different elements (i.e., CHO, CHON, CHOS, CHONS, and CHONSP). We have rearranged Figures 4 and 6 for improved readability and clarity, and optimized the colors and sizes of the legends. The adjustments to the figure captions are highlighted in blue in the revised manuscript.

Thank you very much for your comments and suggestions. Your any further comments and suggestions are appreciated.

Response to Referee 2 (Prof. Maki Teruya):

General comments

R2 Comment 1: This paper reported on the exometabolite (extracellular chemical components) produced by microbial cultures, which are isolated from urban aerosol samples. Investigating the extracellular components from airborne microorganisms are important for understanding the nutrient input by deposition, the survival of airborne microorganisms, the microbial growth, the chemical cycles, the pathogenic strategy and so on. However, I think this paper just showed the measurement data of exometabolite discussing no categorization of ecological means for each isolates producing exometabolite. Additionally, although there are several species of isolates, the exometabolite producing by each species are not categorized and the ecological characteristics of exometabolite have not discussed clearly.

I think this manuscript could be accepted after redrafting the manuscript.

Response:

Dear Prof. Maki Teruya,

Thank you very much for your thorough review and valuable comments on our manuscript. We have carefully considered your suggestions and made corresponding revisions to both the main text and the supplemental information to improve the quality of the manuscript.

We acknowledge your concerns regarding the lack of species-specific categorization of exometabolites and the need for a more explicit discussion of their ecological implications. In response, we have revised the manuscript to provide a more detailed categorization of the exometabolites produced by each isolated species and expanded our discussion on their potential ecological roles. We are confident that these revisions enhance the clarity and depth of the manuscript.

The detailed responses can refer to the point-to-point responses below. Here are the brief responses to your comments.

1) We have classified the bacterial strains into Gram-negative and Gram-positive based on their cellular properties, and the fungal strains into yeasts and molds based on

their growth mode. As a results, all diagrams in the manuscript have been updated to reflect this classification.

2) Additionally, we have restructured the manuscript to focus on two main aspects: (a) the characterization of molecular composition of the exometabolome for different microbial strains and (b) a discussion on the possible ecological roles of these exometabolites.

Some major comments:

R2 Comment 2: I think the total twelve strains are small for discussing the contribution of organic materials to carborne cycles in atmosphere, because there are many kinds of airborne microorganisms, which are unculturable (>90% of microorganisms cannot be cultured but they are living). The authors should discuss deeply on microbial compositions to focus on the dominant species, which can be focused as keystone species.

Response: We acknowledge that a large proportion (over 90%) of airborne microorganisms are unculturable. However, in this study, the bacterial and fungal strains selected in this study represent major taxa commonly found in the atmosphere (refer to Section 3.1). In this presented study, we focused on the dominant culturable species as a first step in understanding their potential contribution to organic material cycling in atmospheric biogeochemical cycle.

In addition to this, we also identified the dominant microbial taxa in aerosols collected at the same sampling site through 16S rRNA high-throughput sequencing and metagenomic analysis (Fig. R1). A detailed characterization of the microbial composition from the same site will be discussed in our further studies.

As shown in Fig. R1, at the genus level, the dominant fungi included *Talaromyces*, *Aspergillus*, and *Penicillium*, which were also cultured and isolated in this study. Similarly, the dominant bacterial genera included *Streptomyces*, *Pantoea*, and *Pseudomonas* (Fig. R1a). At the species level, *Penicillium oxalicum*, *Aspergillus niger*, and *Talaromyces* sp. were among the most abundant taxa, with their relative abundance

ranking within the top 0.5% of all annotated species (Fig. R1b). Additionally, species including *Rhodotorula mucilaginosa*, *Pantoea vagans*, *Bacillus subtilis*, and *Streptomyces pratensis* were also identified, with relative abundances placing them in the top 10% of all detected species. Therefore, the microorganisms selected for our exometabolomic studies indeed represent the dominant atmospheric taxa, ensuring that our findings are relevant to the most prevalent species.

In response to your suggestion, we expanded the discussion to address the broader microbial community, emphasizing the potential ecological roles of the dominant species and their relevance to atmospheric processes. The revised manuscript now reflects these considerations.

Page 7, Line 213 – 217: “The isolated bacterial genera represent major taxa commonly found in the atmosphere (Yan et al., 2021; Lee et al., 2017; Calderon-Ezquerro et al., 2021). Gram-positive bacteria such as *Bacillus* and *Streptomyces* possess cell walls predominantly composed of N-acetylmuramic acid. While, Gram-negative bacteria such as *Pantoea*, *Erwinia*, *Stenotrophomonas*, and *Pseudomonas* have cell walls rich in lipopolysaccharides (LPS)/endotoxin (Ruiz-Gil et al., 2020).”

Page 13, Line 408 – 418: “Most bacterial genera cultured from the atmosphere in various worldwide investigations are Gram-positive bacteria, with *Bacillus* as one of the most abundant genera (Yoo et al., 2019; Maki et al., 2010; Hua et al., 2007). The *Bacillus* genus has often been identified as a dominant taxon in dust aerosols (Rossi et al., 2024; Péguilhan et al., 2023; Maki et al., 2022). For instance, *B. subtilis* has been found to increase significantly during dust events and maintain culturability. *B. subtilis* is known for its high adaptability to diverse extreme conditions, e.g., salt- and temperature-tolerant, allowing it to survive in a wide range of environments (Losick, 2020; Liu et al., 2018; Maki et al., 2022). The versatile metabolic capacity may play a physiological role in facilitating bacterial survival under harsh environments. Previous studies demonstrated that *Bacillus* sp. 3B6 was able to efficiently biotransform sugars into extracellular polymeric substances to protect this bacterium under hostile environment conditions, including cold temperature, radical exposure, and freeze

processes (Matulová et al., 2014). Additionally, members of the *Bacillus* genus are known for their ability to form endospores, enabling them to withstand atmospheric stressors and survive long-range transport (Nicholson et al., 2000).”

Page 14, Line 419 – 427: “*Pantoea* is a highly diverse genus found in both aquatic and terrestrial environments (Walterson et al., 2015; Murillo-Roos et al., 2022). *Pantoea* species exhibit a robust oxidative stress response by synthesizing enzymes such as catalase, superoxide dismutase, and peroxidase (Dahiya et al., 2024; Tambong, 2019). *Pseudomonas* species are well-adapted to atmospheric conditions, and they often oxidize common atmospheric volatile organic compounds, including formaldehyde and methanol (Husarova et al., 2011). *Streptomyces* is a dominant genus in aerosols from various megacities and background areas, especially in winter (Chen et al., 2021a; Petroselli et al., 2021; Li et al., 2019). Furthermore, *Streptomyces* has been widely reported in aerosols from European deserts and urban sewage treatment plants (Núñez et al., 2024; Zhan et al., 2024). *Streptomyces* is a drought-tolerant bacterial genus with spore-forming ability (Taketani et al., 2016). Their spores can enter a stable and quiescent state under environmental stress, enabling survival in diverse natural conditions (Naylor et al., 2017).”

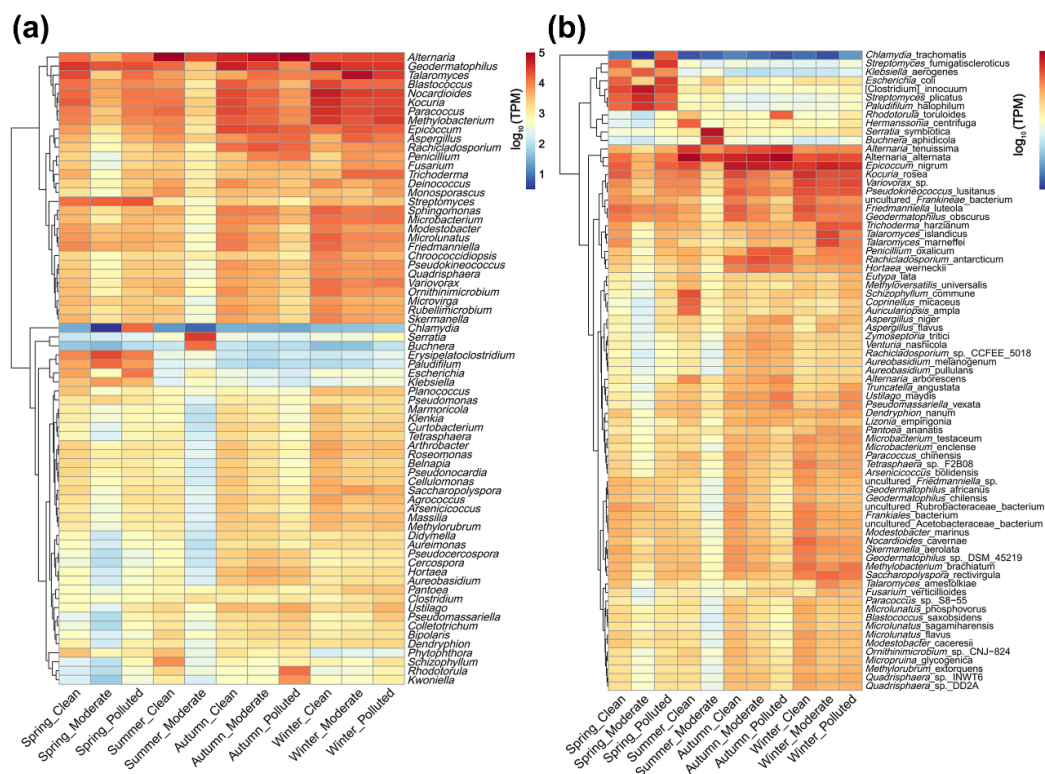


Figure R1 Characterization of atmospheric microbial composition in urban Tianjin. (a) Microbial composition at the genus level with the transcript per kilobase per million mapped reads (TPM) greater than 1000 reads; (b) Microbial composition at the species level with the TPM greater than 500 reads (top 0.5%) (unpublished data).

R2 Comment 3: Extracellular products are composed of several kinds of chemical components, which contribute to the roles of airborne microorganisms. Accordingly, after the categories of ecological merits by the chemical components are defined, the categories of chemical components are established for understanding the roles of extracellular products on airborne bacterial ecology. For examples, there are the some categories such as 1) Survivals of airborne microorganisms in atmosphere, 2) Microbial growth in atmosphere and after deposition, 3) Chemical cycles in several environments, and 4) Pathogenic abilities using some components and so on.

Response: Thank you for your insightful comments. We fully agree that categorizing the ecological roles of extracellular products based on their chemical components is crucial for a deeper understanding of airborne microorganisms' ecology. In response, we have revised the manuscript to better define the ecological significance of the chemical components produced by the isolated microorganisms. Importantly, we have highlighted certain interesting products and discussed their potential ecological functions, mainly in the Results or Discussion sections.

These revisions are integrated throughout several paragraphs in the manuscript. All changes are clearly marked in blue in the revised manuscript.

The main revisions include:

- 1) Page 14, Line 429 – 436: “Specifically, the exometabolites of *Penicillium* and *Talaromyces* were enriched with pigment compounds, particularly CRAM-like molecules with CHO elemental compositions (Fig. 6a and 6b). Some aliphatic/peptide-like and aromatic-like compounds were biotransformed into CRAM-like pigment molecules ($C_{14}H_{10}O_6$, $C_{23}H_{24}O_7$, $C_{26}H_{29}NO_8$, $C_{14}H_{10}O_6$, and $C_{15}H_{10}O_5$) (Fig. 7c and 7d). Microbial pigments are chemically diverse, including

flavonoids, isoprenoids, porphyrins, N-heterocyclics, and polyketides (Contreras-Machuca et al., 2022; Morales-Oyervides et al., 2020; Venkatachalam et al., 2018). Recent studies have highlighted the potential of *Penicillium* and *Talaromyces* as robust producers of natural pigments, noted for their thermal, pH, and light stability (Ugwu et al., 2021; Akilandeswari and Pradeep, 2016)."

- 2) Page 15, Line 481 – 487: "A large number of CHONS compounds, belonging to aliphatic/peptide-like molecules, were detected in the metabolites of the Gram-negative bacteria *Stenotrophomonas* sp. and *P. baetica*, accounting for 40.6% (55.3% of intensity) and 33.2% (61.8% of intensity), respectively (Fig. 4b and S5). These compounds, including extracellular polymeric substances, play a crucial role in microbial survival by forming protective biofilms and contributing to the atmospheric biogeochemical cycle. The most frequently detected genus in the atmosphere, *Pseudomonas*, is a key producer of biosurfactants, which can impact atmospheric chemistry (e.g., secondary organic formation) and modify cloud microphysics by enhancing cloud condensation nuclei activation owing to their exceptional ability to reduce surface tension (Delort et al., 2010)."
- 3) Page 16, Line 488 – 496: "Building on prior studies, some *Stenotrophomonas* spp. are known to synthesize a range of biologically active compounds, including a variety of antibiotic enzymes such as β -lactams, aminoglycosides, and macrolides, alongside chitinases, lipases, and proteases (Ryan et al., 2009; Peleg and Abbott, 2015; Wang et al., 2018). These findings suggest that many of the CHONS compounds identified in the exometabolites are likely proteases or their degradation products, underscoring the pivotal role of *Stenotrophomonas* in protein turnover and nutrient cycles in the land/water-air interfaces (Liu et al., 2016). Furthermore, the molecular composition of the exometabolites pointed to an enrichment of amino acid metabolic pathways, like tyrosine metabolism (KO00350) and histidine metabolism (KO00340) (Fig. 8a). These pathways, coupled with the known reactivity of amino acids, highlight the importance of considering the amino acid presence and transformation when investigating the chemical composition and

oxidative processes occurring within cloud water (Bianco et al., 2016).”

- 4) Page 16, Line 502 – 510: “*R. mucilaginosa* was predominantly involved in carbohydrate metabolism (Fig. 8b). In this study, several saturated fatty acids, including myristic acid ($C_{14}H_{28}O_2$), stearic acid ($C_{18}H_{36}O_2$), and palmitic acid ($C_{16}H_{32}O_2$), and unsaturated fatty acids including linoleic acid ($C_{18}H_{32}O_2$) and linolenic acid ($C_{18}H_{30}O_2$), were detected. These organic acids were also abundantly present in atmospheric aerosols and may affect the atmospheric chemical and physical processes, e.g., cloud formation (Mkoma and Kawamura, 2013; Balducci and Cecinato, 2010; Raymond and Pandis, 2002). Notably, palmitic acid, stearic acid, and linoleic acid are potential molecular tracers for estimating the contribution of cooking emissions to organic aerosols (Cheng and Yu, 2020; Ma et al., 2023). However, our findings reveal that fatty acids were also abundant in the metabolites of *Rhodotorula*, suggesting that microorganisms may contribute to atmospheric fatty acid levels. This highlights the need to consider microbial sources when assessing the composition of atmospheric organic aerosols.”
- 5) Page 16, Line 511 – 524: “Among airborne fungi, the genera *Aspergillus*, *Penicillium*, and *Talaromyces*, which are classified as mold fungi, are acknowledged as some of the most chemically prolific organisms. These genera can synthesize a wide variety of secondary metabolites (exometabolites) (Frisvad, 2015; Zhai et al., 2016; Morales-Oyervides et al., 2020). They produce specialized compounds such as polyketides, non-ribosomal peptides, and terpenes, further showcasing their metabolic versatility and biochemical complexity (Frisvad, 2015; Adelusi et al., 2022). This remarkable chemodiversity highlights their ecological significance in atmospheric biogeochemistry. *A. niger* is ubiquitous in the environment and plays a significant role in the global carbon cycle (Baker, 2006; Schuster et al., 2002; Šimonovičová et al., 2021). It is rich in genetic and metabolic diversity, acting as one of the most important microorganisms used in biotechnology (Sun et al., 2007; Jørgensen et al., 2011). In its natural growth state, *A. niger* possesses large cryptic biosynthetic gene clusters (BGCs), which

synthesize a wide range of extracellular enzymes to degrade special biopolymers in the environment, thus allowing the fungus to obtain nutrients (Yu et al., 2021; Romsdahl and Wang, 2019). Anthraquinones like C₁₅H₁₂O₅, C₁₅H₁₀O₅, and C₁₅H₁₀O₆ were detected in the product of *Penicillium* and *Talaromyces*, and this compound can protect symbiotic plants from pests or pathogens (Etalo et al., 2018). This metabolic plasticity underscores their ecological importance and the potential for biotechnological applications in various industries.”

R2 Comment 4: The six isolates of bacteria or fungi can be classified to some groups in dependence on the taxon. The chemical components in extracellular products are discussed for each category to understand the ecological characteristics of each categorized microorganisms. This comments are relating to Comment 1.

Response: We revised the manuscript to include a more detailed categorization of the exometabolites produced by each isolated species and expanded the discussion on their potential ecological roles. We have classified bacterial strains into Gram-negative and Gram-positive based on their cellular properties, and fungal strains into yeasts and molds based on their growth modes. These revisions are all marked in blue in the revised manuscript.

The main revisions include:

- 1) Page 9, Line 285 – 293: “*Stenotrophomonas* sp. and *P. baetica*, both Gram-negative bacteria, metabolized and synthesized large quantities of CHONS compounds (55.3–61.8% of intensity), largely differed from the other four bacteria strains (only 3.5–21.5% of intensity). Notably, in exometabolites from *Stenotrophomonas* sp., CHONS compounds dominated the lipid-like and aliphatic/peptide-like molecules, distinguishing it from the other bacterial strains (Fig. 4b). Microbes can convert bioavailable lipid-like and protein-like nitrogenous compounds into more oxygenated, unsaturated (more refractory) nitrogenous CRAM/lignin-like compounds (Osborne et al., 2013). Additionally, *Stenotrophomonas* sp. produced a higher number (903 formulas) of high molecular weight compounds ($m/z > 500$)

(Table S6), likely representing extracellular polymeric substances, e.g., polysaccharides, extracellular enzymes, and cellular debris (Vandana et al., 2023; Moradali and Rehm, 2020)."

- 2) Page 10, Line 305 – 311: "For Gram-positive bacteria, the elemental compositions of the unique formulas were similar, with CHON compounds accounting for more than 50% of all compounds (Fig. 5a). In contrast, for Gram-negative bacteria, CHON compounds were predominant (48.1%) in the unique formulas specific to *P. vagans*, and CHONS compounds are predominantly those specific to *Stenotrophomonas* sp. and *P. baetica*, with proportions of 70.2% and 71.4%, respectively (Fig. 5a). In addition, CHO compounds comprised over 25% of the unique formulas found exclusively in *P. vagans* (Fig. 5a). Notably, among these unique CHO compounds, the most abundant formulas belonged to the O₉ class, dominated by C₁₈H₂₈O₉ and C₁₃H₁₆O₉, indicating higher oxidation states (Fig. S5c)."
- 3) Page 10, Line 319 – 324: "Unlike the bacterial strains, which all produced high fractions of CHONS compounds, CHONS compounds appeared only in exometabolites of *R. mucilaginosa*, a yeast species, accounting for approximately 19% of the total formula number (Fig. 6a). This indicates a diversity of sulfur-related metabolic processes for *Rhodotorula*. In contrast, the exometabolites produced by mold fungi, specifically from the genera *Penicillium* (*Penicillium oxalicum* and *Penicillium aurantiogriseum*) and *Talaromyces* (*Talaromyces* sp.), were predominantly CHO compounds, accounting for over 50% of the total exometabolite intensity (Fig. 6a)."
- 4) Page 15, Line 481 – 487: "A large number of CHONS compounds, belonging to aliphatic/peptide-like molecules, were detected in the metabolites of the Gram-negative bacteria *Stenotrophomonas* sp. and *P. baetica*, accounting for 40.6% (55.3% of intensity) and 33.2% (61.8% of intensity), respectively (Fig. 4b and S5). These compounds, including extracellular polymeric substances, play a crucial role in microbial survival by forming protective biofilms and contributing to the atmospheric biogeochemical cycle. The most frequently detected genus in the

atmosphere, *Pseudomonas*, is a key producer of biosurfactants, which can impact atmospheric chemistry (e.g., secondary organic formation) and modify cloud microphysics by enhancing cloud condensation nuclei activation owing to their exceptional ability to reduce surface tension (Delort et al., 2010)."

R2 Comment 5: The Results and Discussion should be separated each, because there are many overlapped parts and redundancy, which make readers understand this hardly.

Response: Thank you for your valuable comments. To enhance the readability of the paper, we have separated and reorganized the Results and Discussion sections according to the specific findings and conclusions. All corrections are marked in the revised manuscript.

R2 Comment 6: I think the authors are not familiar with biology. So some biologist suggestion is needed for categorizing the taxon and describing the taxon names.

Response: Thank you for your comments. We are not sure whether do you mean we should designate the name of microbial strains by using letters, symbols, or numbers, and use the new names of bacterial phyla standardized in the year of 2021. For the former one, we renamed the strains using TJB1–24 and TJF1–16 in Table S2, S3, S4. But for concision, we use species names instead of strains names in the main text and figures. For the latter one, we have checked and used the new taxon names in the manuscript and Figure 1. The corrections are marked in blue in the revised manuscript.

Some minor comments:

R2 Comment 7: L19: What is CHON?

Response: According to the elemental composition of organic compounds, their molecular composition is classified into four categories: CHO, CHON, CHOS, and CHONS. This classification has been commonly used in mass spectrometry analyses. For example, CHON means that the organic compounds contain only four elements: carbon, hydrogen, oxygen, and nitrogen without any other elements.

In the revision, we added “For example, CHO indicates that the organic compounds in this category contain only three elements: carbon, hydrogen, oxygen, with no other elements.” in Line 198 – 199, Page 7.

R2 Comment 8: L22: Fungi also produce amino acid.

Response: We complemented this sentence by integrating the results presented in Fig. 8 (b). The revision has been made and is marked in blue (see the annotated revision).

Page 1, Line 24 – 25: “Bacteria exhibited proficiency in amino acid synthesis, while fungi were actively involved in amino acid metabolism, transcription, and expression processes.”

R2 Comment 9: L25-L28: This is general summary. Please summarize specific topics for this study.

Response: We understand the importance of providing a more focused summary highlighting the specific topics of our study. We revised the summary to emphasize the main objectives and findings of our study, which will be more explicitly detailed in the revised manuscript to provide a clearer understanding of the study’s scope and contributions.

Page 1, Line 27 – 29: “This study provides new insights into the transformation and potential oxidative capacity of atmospheric microorganisms concerning organic matter at air-land/water interfaces. These findings are pivotal for assessing the biogeochemical impacts of atmospheric microorganisms following their deposition.”

R2 Comment 10: L30-L40: The sampling is performed in Asian site. The papers relating to Asian-dust transport of bioaerosols are needed. I think European bioaerosols are different from Asian bioaerosols. So the author can isolate the *Bacillus* strain efficiently.

Response: Thank you for your comments. We acknowledge the importance of including literature on the transport of bioaerosols associated with Asian dust events.

We have incorporated relevant references to provide a more comprehensive context. Regarding the comparison between European and Asian bioaerosols, we agree that geographical differences may influence microbial composition. However, *Bacillus* is also illustrated as the dominant bacterial genus in many studies on the composition of European bioaerosol bacterial communities (Rossi et al., 2024; Péguilhan et al., 2023; Chatoutsidou et al., 2023; Nunez et al., 2021). For example, the relative abundance of *Bacillus* was even 13% among atmospheric bacteria at the Puy de Dôme observatory in France. Our study focused on dominant taxa found in the atmospheric samples, with *Bacillus* being one of the primary subjects. We appreciate your suggestion and have incorporated a discussion on the specificity of bioaerosols across different regions into the revised manuscript.

- 1) Page 13, Line 408 – 418: “Most bacterial genera cultured from the atmosphere in various worldwide investigations are Gram-positive bacteria, with *Bacillus* as one of the most abundant genera (Yoo et al., 2019; Maki et al., 2010; Hua et al., 2007). The *Bacillus* genus has often been identified as a dominant taxon in dust aerosols (Rossi et al., 2024; Péguilhan et al., 2023; Maki et al., 2022). For instance, *B. subtilis* has been found to increase significantly during dust events and maintain culturability. *B. subtilis* is known for its high adaptability to diverse extreme conditions, e.g., salt- and temperature-tolerant, allowing it to survive in a wide range of environments (Losick, 2020; Liu et al., 2018; Maki et al., 2022). The versatile metabolic capacity may play a physiological role in facilitating bacterial survival under harsh environments. Previous studies demonstrated that *Bacillus* sp. 3B6 was able to efficiently biotransform sugars into extracellular polymeric substances to protect this bacterium under hostile environment conditions, including cold temperature, radical exposure, and freeze processes (Matulová et al., 2014). Additionally, members of the *Bacillus* genus are known for their ability to form endospores, enabling them to withstand atmospheric stressors and survive long-range transport (Nicholson et al., 2000).”
- 2) Page 14, Line 423 – 427: “*Streptomyces* is a dominant genus in aerosols from

various megacities and background areas, especially in winter (Chen et al., 2021a; Petroselli et al., 2021; Li et al., 2019). Furthermore, *Streptomyces* has been widely reported in aerosols from European deserts and urban sewage treatment plants (Núñez et al., 2024; Zhan et al., 2024). *Streptomyces* is a drought-tolerant bacterial genus with spore-forming ability (Taketani et al., 2016). Their spores can enter a stable and quiescent state under environmental stress, enabling survival in diverse natural conditions (Naylor et al., 2017).”

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R2 Comment 11: L64-L65: This is not related to the organic matters.

Response: We have deleted these sentences and integrated this part into the relevant part of the introduction (Page 2, Line 52 – 54 and Page 3, Line 86 – 88).

R2 Comment 12: L83-L90: This sampling site is located in Asia. How is Asian dust events?

Response: Thank you for your comments. This study only provides a preliminary result on the potential transformation and potential oxidative capacity of culturable urban atmospheric microorganisms for organic matter based on typical microbial strains, without considering different types of aerosols.

During the sampling period in this study, Asian dust events were not recorded. While, we are utilizing exometabolome assays and data analysis methods from this study to investigate the activity and metabolic capacity of atmospheric microorganisms following the deposition of Asian dust into surface seawater. We have conducted a thorough examination of these microorganisms' responses and are currently compiling and analyzing the specific findings. The related results will be published in future work.

R2 Comment 13: L91-L101: The analytical methods should be the results relating to more ecological topics. I think this section just show analytical method index.

Response: Thank you for your comments. We understand your concern regarding the need for the analytical methods to be more closely related to ecological topics. In this section, we intend to present the analytical methods and demonstrate their validity, which is why it may appear more focused on methodology. We have condensed this section and added some applications in ecology.

Page 3, Line 85 – 96:

“Microbial exometabolites hold great ecological significance, as they can serve as substrates for other microorganisms, facilitating population growth (Douglas, 2020). For instance, the metabolome of *Pseudomonas graminis* exposed to oxidative stress involves carbohydrate, glutathione, energy, lipid, peptide, and amino acid metabolism pathways (Wirgot et al., 2019). Furthermore, microbial exometabolites, including extracellular polymeric substances and pigments, are vital for the survival of atmospheric microorganisms, enabling them to withstand long-range transport and extreme conditions in the atmosphere or at the ocean surface (Erkorkmaz et al., 2023;

Bryan et al., 2019). Recently, a framework for atmo-ecometabolomics, from sampling to data analysis, was developed to characterize the molecular composition of aerosols (Rivas-Ubach et al., 2019). This framework also provides insights into how aerosol chemical compositions impact ecosystem structure, function, and biogeochemistry. However, previous studies have not fully explored the role of metabolically active microorganisms in atmospheric aerosols. Deepening our understanding of the metabolome of atmospheric microorganisms is essential for predicting their ecological roles in biogeochemical cycles and assessing their broader impacts on ecosystem functions and atmospheric processes.”

R2 Comment 14: L110: I do not think KEGG analysis is needed for this paper. However, if KEGG analysis is remained, the KEGG analysis should be introduce in the introduction section.

Response: Thank you for your comments. We have now incorporated the KEGG explanation into the introduction section, with these revisions marked in blue in the revised manuscript. We chose to use KEGG analysis because it allows us to comprehensively explore the biochemical reaction processes that are highly relevant to atmospheric microorganisms. The metabolome provides a dynamic snapshot of an organism’s metabolic state, capturing the complex interactions among genes, proteins, and other factors. By utilizing KEGG identifiers for the metabolites we identified, we could map these against bioinformatic databases using the KEGG mapper in batch mode, systematically identifying and analyzing the involved biochemical pathways. This approach offers a robust framework for understanding the complex metabolic functions and key shifts in microbial processes, helping to elucidate the unique metabolic processes that support microbial survival and adaptation in atmospheric environments.

Page 4, Line 101 – 108:

“Relevant biosynthetic pathways can be traced through chemical information, relying on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Lopez-

Ibañez et al., 2021). KEGG is a comprehensive database resource designed to facilitate interpreting high-level functions and systems by integrating molecular-level data, including cellular, organismal, and ecological processes. This database is particularly valuable for analyzing large-scale molecular datasets derived from genomic sequencing and other high-throughput technologies, enabling the exploration of complex biological pathways and networks (Kanehisa et al., 2017). Biochemical pathways were identified by mapping the molecular composition of metabolites obtained by FT-ICR MS to chemical structures in the KEGG COMPOUND Database using the analytical pipeline developed by Ayala-Ortiz et al. (2023).”

R2 Comment 15: L123: How many samples are collected? The sample numbers should be describe and the samples for each isolates are explained. Additionally, the environmental factors have to be discussed when the isolates are obtained.

Response: Thank you for your thoughtful suggestion. Regarding the experimental design, we conducted our experiment with three samples, each analyzed in parallel with three replicates. While we acknowledge that the sample size may be somewhat limited, we carefully distinguished different microbial phenotypes during the isolation process. Notably, the microorganisms selected for our exometabolomic studies represent the dominant taxa of atmospheric microorganisms, ensuring that our findings are relevant to the most prevalent species. Additionally, we would like to clarify that the primary aim of our research was to investigate the potential metabolic capacities of these atmospheric microorganisms, rather than the influence of environmental factors. Nonetheless, we highly value your suggestions and will certainly incorporate these considerations into future studies. We have added relevant notes about the samples to the revised manuscript.

Page 5, Line 129 – 134:

“Each sample underwent three parallel experiments. Microorganisms on the filters were detached using low-power ultrasonication in an ice bath for 5 min and then centrifuged at 250 rpm for 30 min. Then, a 100 µL aliquot of the well-mixed suspension

was spread-plated onto the two types of solid media. For each suspension, three independent replicates were prepared to ensure that both media were thoroughly coated and evaluated. The plates were incubated at a constant temperature of 37°C for 48 h for bacteria, and at 28°C for 72 h for fungi, with daily growth monitoring (Wang et al., 2023; Timm et al., 2020)."

R2 Comment 16: L184-L185: The chemical components should be defined in detail, because these are familiar with the readers for ACP.

Response: We added the meanings of these chemical components and labeled them in blue.

Page 7, Line 198 – 199:

"For example, CHO indicates that the organic compounds in this category contain only three elements: carbon, hydrogen, and oxygen, with no other elements."

R2 Comment 17: L185: KEGG explanation is needed in the Introduction. Why was this used for this paper?

Response: Thank you for your comments. We have now incorporated the KEGG explanation into the introduction section, with these revisions marked in blue in the revised manuscript. We chose to use KEGG analysis because it allows us to comprehensively explore the biochemical reaction processes that are highly relevant to atmospheric microorganisms. The metabolome provides a dynamic snapshot of an organism's metabolic state, capturing the complex interactions among genes, proteins, and other factors. By utilizing KEGG identifiers for the metabolites we identified, we could map these against bioinformatic databases using the KEGG mapper in batch mode, systematically identifying and analyzing the involved biochemical pathways. This approach offers a robust framework for understanding the complex metabolic functions and key shifts in microbial processes, helping to elucidate the unique metabolic processes that support microbial survival and adaptation in atmospheric environments.

Page 4, Line 101 – 108:

“Relevant biosynthetic pathways can be traced through chemical information, relying on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Lopez-Ibañez et al., 2021). KEGG is a comprehensive database resource designed to facilitate interpreting high-level functions and systems by integrating molecular-level data, including cellular, organismal, and ecological processes. This database is particularly valuable for analyzing large-scale molecular datasets derived from genomic sequencing and other high-throughput technologies, enabling the exploration of complex biological pathways and networks (Kanehisa et al., 2017). Biochemical pathways were identified by mapping the molecular composition of metabolites obtained by FT-ICR MS to chemical structures in the KEGG COMPOUND Database using the analytical pipeline developed by Ayala-Ortiz et al. (2023).”

R2 Comment 18: L193: As described, I recommend to separate to each section of Result and Discussion.

Response: To enhance the readability of the paper, we have separated and reorganized the Results and Discussion sections according to the specific findings and conclusions. All corrections are marked in the revised manuscript.

R2 Comment 19: L196-L197: The strain numbers should be described.

Response: We double-checked the content in Line 196 – 197 (in the original manuscript), which are part of the description of the isolation results, mainly describing the dominant taxa of culturable bacteria at the phylum and genus levels. It does not address specific strains or strain numbers.

R2 Comment 20: L262: The genus name should be initial after second using. For example, *A. niger*. This is biological rule.

Response: Thank you for your comments. We have corrected the microbial names following this rule throughout the manuscript. The corrections are labeled in blue.

R2 Comment 21: Fig.1. The new phyla name of bacteria should be used.

Response: Thank you for your comments. We modified the bacterial phylum names in Figure 1, as well as in the main text and supplement (Table S2), to the new names standardized in the year of 2021.

Page 7, Line 211 – 212: “The culturable bacterial community primarily consisted of Pseudomonadota (45.8%), Bacillota (37.5%), and Actinomycetota (16.7%).”

R2 Comment 22: L362-L430: This section is overlapped to Section 3.2. Please combine them.

Response: Thank you for your comments. We understand your concern regarding the potential overlap between these sections. However, the structure of our article is intentionally designed to first present the molecular characterization of the exometabolites (Sect. 3.2 and 3.3), followed by an analysis of the metabolic processes (Sect. 3.4). This sequence allows us to discuss the metabolic processes directly related to the identified products, ensuring a more coherent flow of information. By organizing the sections in this way, we align to corroborate the identified metabolites with the metabolic pathways responsible for their production, providing a more comprehensive understanding of the data. We have made the necessary changes in the manuscript and hope that this clarifies the structure of our article.

Thank you very much for your comments and suggestions. Your any further comments and suggestions are appreciated.

Response to Referee 3:

In this study, the authors examined the microbial exometabolism production of culturable airborne microorganisms from an urban atmosphere. A diverse range of products, including CHON, CHONS, and CHO compounds, were identified for both bacteria and fungi. Significant variations in metabolites were observed among different strains. In terms of amino acid synthesis, transcription and expression processes, lipid metabolism, amino acid metabolism, and carbohydrate metabolism, there was a wide variation among bacterial and fungal species. The results provide a comprehensive examination of metabolite characteristics at the molecular level for typical culturable airborne microorganisms. Overall, this work is novel and interesting, and the ideas and data presented in the manuscript are credible. This paper could be accepted after appropriately revision. More detailed comments/suggestions are provided below.

R3 Comment 1: L78 The influence of dry deposition dust on marine terrestrial ecological environments was discussed, with a particular focus on microbial communities. Additionally, the effects of wet deposition, including cloud, fog, rain, or snow processes should clarify.

Response: Thank you for your comments. We added the impact of wet deposition on marine ecosystems in the introduction section, which is marked in blue in the revised manuscript.

Page 3, Line 73 – 80: “Wet deposition (e.g., rainfall) brings a wealth of nutrients and microorganisms to surface waters, increasing chlorophyll *a* concentration and enhancing carbon and nitrogen fixation. Short-term wet deposition events play an important role in the temporal variation of new nitrogen production and phytoplankton dynamics in the southeastern Mediterranean, as demonstrated by both experimental and in situ observations (Rahav et al., 2021). Additionally, one notable study demonstrated that rainwater influenced by Saharan dust significantly increased bacterial populations in high-altitude lake water, with rare taxa becoming dominant based on cultivation experiments (Peter et al., 2014). Despite these findings, there is a lack of research on

how atmospheric microorganisms metabolize and degrade organic matter, making it challenging to accurately assess their metabolic processes and potential impacts on atmospheric chemistry and biogeochemical cycles.”

R3 Comment 2: Line 102-109, this section is an introduction to the FT-ICR MS method, which could be integrated into section 2. Materials and methods.

Response: We have reorganized the introduction section and moved the part introducing FT-ICR MS to the Materials and methods section. The revisions have been highlighted in blue in the revised manuscript.

Page 6, Line 184 – 185: “Since FT-ICR MS determines potent polarity molecules with molecular weight (MW) from 100 Da to 1000 Da, pigments or extracellular enzymes secreted by bacteria or fungi can be detected and identified.”

R3 Comment 3: Line 123, the sampling period is three days, yet it remains unclear whether the sample volume satisfies the prerequisites for analysis. Could you elucidate the criteria employed to determine the sampling dates? Were these days being clean, polluted, or sunny or rainy/snowy days? Furthermore, could you elaborate on why the sampling time was specifically set between 15:00-19:00? Please offer further insights into the sampling procedure.

Response: Thank you for your comments. Our exploratory study specifically focused on culturable atmospheric microorganisms which are prevalent and highly active, as well as their potential effects on atmospheric biogeochemistry. Therefore, we did not extensively consider the effects of weather conditions on the isolation and cultivation of atmospheric microorganisms. The weather conditions and air quality during the sampling periods are summarized in Table R1, showing air quality levels that ranged from good, moderate, to lightly polluted, representing typical winter conditions.

Regarding the sampling time (15:00 – 19:00), we randomly selected this period to capture the dominant taxa prevalent among atmospheric microorganisms. Unfortunately, however, we could not document significant differences in isolates

among the three samples. This exploratory study has elucidated the metabolic activity of atmospheric microorganisms and the molecular composition of their exometabolites, providing a basis for investigating their potential role in biogeochemical cycles, particularly the carbon cycle.

We thank you for your suggestions and will consider this in our subsequent studies.

Table R1 Weather conditions and air quality during the sampling periods.

Date	Temperature (°C)	Weather	AQI	Air quality level	PM _{2.5}	PM ₁₀	SO ₂	NO ₂	CO	O ₃
2022.01.05	-4 – 4	fine	68	Moderate	39	71	11	54	1	29
2022.01.08	-2 – 9	fine	125	Lightly polluted	95	129	7	53	1.2	48
2022.01.11	-5 – 1	cloudy	40	Good	12	25	9	32	0.7	48

AQI: Air Quality Index; Unit of value: $\mu\text{g}/\text{m}^3$ (mg/m^3 for CO)

R3 Comment 4: Line 132, the methodology for microbial culture and separation must be detailed, including specific parameters such as centrifugal speed and the volume of bacteria collected.

Response: We added further details on the microbial culture and isolation processes in the revised manuscript.

Page 5, Line 134 – 144: “From each plate, all phenotypically distinct colonies were picked up and smeared onto fresh media for isolation. Single colonies were picked and re-streaked from each plate at least three times to isolate individual strains (Timm et al., 2020). Multiple streaking isolations are essential to obtain stable and pure strains by gradually diluting the microbial population and allowing for the isolation of individual colonies (Yan et al., 2021; Dunbar et al., 2015).

Isolated strains were preserved using the cryopreservation method. Bacterial strains were cultured in tryptic soy broth (TSB) at 37°C with shaking at 200 rpm, while fungal strains were cultured at 28°C on SDA medium plates under a stationary condition. When the bacteria reached the logarithmic phase, 750 μL of the culture was mixed thoroughly with an equal volume of 50% glycerol, resulting in a final glycerol concentration of 25%, and then stored at -80°C . For fungal strains, once spores were

evenly distributed across the plate, they were eluted with 4 mL of sterile PBS buffer. A 750 μ L aliquot of the spore suspension was then mixed with an equal volume of 50% glycerol and stored at -80°C with a final glycerol concentration of 25%.”

Page 5, Line 174 – 178: “The metabolic products (2 mL) filtered by 0.22 μm pore membranes were acidified to pH 2 using high-pressure liquid chromatography (HPLC) grade hydrochloric acid (HCl). Dissolved organic matter (DOM) was extracted using a solid phase extraction (SPE) cartridge (200 mg, Oasis HLB, 6cc, Waters, U.S.) to remove salts (Chen et al., 2022; Han et al., 2022). After extraction, the cartridges were dried by flushing with high-purity N_2 . Finally, 6 mL of HPLC-grade methanol (Sigma-Aldrich) was used to elute the extracted DOM. A 2 mL aliquot of the eluent was collected and stored at -20°C for further analysis.”

R3 Comment 5: Line 158-160, the culture time of bacteria is 7 days, and fungi is 15 days. Is there any reference for the culture time? Or refer to the residence time of microorganisms in the atmosphere? In Line 241, whether the results of different times are comparable compared to the results of culture for 30 days?

Response: Thank you for your comments. The choice of culture time was mainly based on the literatures on the viability and metabolic activities (e.g., organic biotransformation) of airborne bacteria and fungi, as well as their residence time in the atmosphere. Most experimental designs studying bacterial metabolites typically use an incubation time of 3 to 10 days. For example, Matulová et al. (2014) investigated the metabolic ability of *Bacillus* sp. 3B6, culturing the bacterial strain for 5 – 8 days to degrade different types of sugars and produce extracellular polymeric substances (EPSs). Similarly, Na et al. (2023) selected a 7-day incubation time to study the viability of atmospheric bacteria.

In addition, the kinetic study in microbial degradation of atmospheric organic compounds showed that about 15 days of incubation were required for the complete conversion of dicarboxylic acids (DCA) by fungi (Ariya et al., 2002). Some other studies on fungal metabolites used 10 – 20 days for fungal liquid fermentation (Woo et

al., 2014; Adelusi et al., 2022).

On the other hand, evidences suggest that atmospheric bacteria can reside for 2–15 days, enabling potential intercontinental transport of airborne bacteria (Lappan et al., 2024; Šantl-Temkiv et al., 2022). Laboratory simulations have further illustrated that both airborne bacteria and fungi maintain biological effectiveness for about 20 days, regardless of air pollution (Xu et al., 2021).

Considering these findings, we selected an experimental protocol of 7 days for bacterial incubation and 15 days for fungal incubation. Although incubation times vary across studies, microbial metabolic activities are continuous and dynamic processes. During these incubation periods, bioavailable organic molecules can undergo sufficient transformation, allowing the comparisons between different results meaningful.

References:

Adelusi, O. A., Gbashi, S., Adebisi, J. A., Makhuvele, R., Adebo, O. A., Aasa, A. O., Targuma, S., Kah, G., and Njobeh, P. B.: Variability in metabolites produced by *Talaromyces pinophilus* SPJ22 cultured on different substrates, *Fungal Biology and Biotechnology*, 9, <https://doi.org/10.1186/s40694-022-00145-8>, 2022.

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Woo, P. C. Y., Lam, C.-W., Tam, E. W. T., Lee, K.-C., Yung, K. K. Y., Leung, C. K. F., Sze, K.-H., Lau, S. K. P., and Yuen, K.-Y.: The biosynthetic pathway for a thousand-year-old natural food colorant and citrinin in *Penicillium marneffei*, Scientific Reports, 4, <https://doi.org/10.1038/srep06728>, 2014.

Xu, C., Chen, H., Liu, Z., Sui, G., Li, D., Kan, H., Zhao, Z., Hu, W., and Chen, J.: The decay of airborne bacteria and fungi in a constant temperature and humidity test chamber, Environment International, 157, 106816, <https://doi.org/10.1016/j.envint.2021.106816>, 2021.

R3 Comment 6: Line 198, here discussed the genus of *Pantoea*, is *Pantoea* also a dominant bacterium? Another predominant strain, *Streptomyces*, was not further explained.

Response: Bacteria of the genus *Pantoea* were successfully isolated and characterized in our culture experiments, demonstrating the airborne bacterial cells were intact, viable, and culturable. In our ongoing studies, we analyzed the atmospheric bacterial community during the winter season in urban Tianjin through metagenomic sequencing. The results showed that *Pantoea* was the major bacterial taxa among the atmospheric bacteria (Fig. R2). Moreover, *Pantoea* is a well known genera containing potential ice-nucleating active bacterial species (Akila et al., 2018; Koda et al., 2000; Muryoi et al., 2003). Therefore we discussed the genus *Pantoea* in this paper. Additionally, we added a discussion of the dominant bacterium, *Streptomyces*, highlighted in blue in the revised manuscript.

Page13, Line 403 – 407: “At the genus level, culturable atmospheric bacteria isolated from the aerosol samples mainly included *Bacillus*, *Pseudomonas*, *Streptomyces*, and *Pantoea* (Fig. 1). These bacterial taxa were predominant across urban, rural and forest aerosols, displaying strong environmental adaptability (Nunez et al., 2021; Gusareva et al., 2019; Souza et al., 2021). Their widespread distribution suggests remarkable resilience to harsh environmental stressors, enabling them to survive and maintain culturability (Hu et al., 2018; Hu et al., 2020).”

Page 14, Line 423 – 427: “*Streptomyces* is a dominant genus in aerosols from various megacities and background areas, especially in winter (Chen et al., 2021a; Petroselli et al., 2021; Li et al., 2019). Furthermore, *Streptomyces* has been widely reported in aerosols from European deserts and urban sewage treatment plants (Núñez et al., 2024; Zhan et al., 2024). *Streptomyces* is a drought-tolerant bacterial genus with spore-forming ability (Taketani et al., 2016). Their spores can enter a stable and quiescent state under environmental stress, enabling survival in diverse natural conditions (Naylor et al., 2017).”

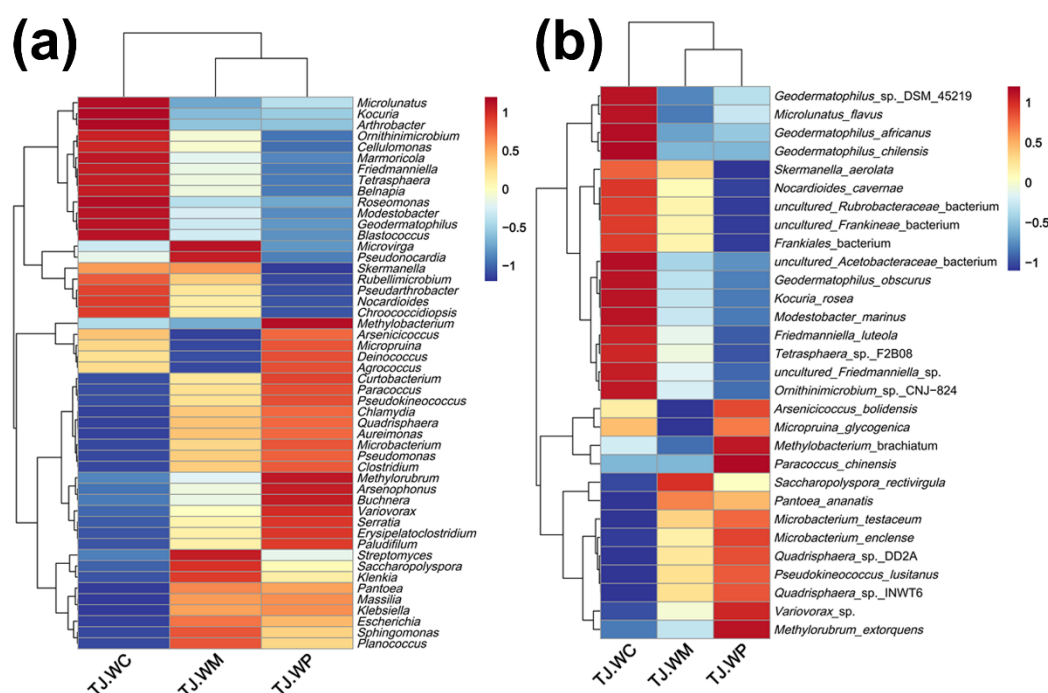


Figure R2 Relative abundance of bacterial composition during winter in urban Tianjin (TJ). (a) Bacterial composition at the genus level showing the top 50 genera; (b) Bacterial composition at the species level showing the top 30 species. WC, WM, and WP mean a sample collected on clean, moderate polluted, polluted winter days, respectively (unpublished data).

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R3 Comment 7: Line 279, the diversity of exometabolomic products in bacteria and fungi is not analogous, hence a comparison between the two is not advisable. However, it is permissible to separately compare bacterial diversity across different samples and fungal diversity across different samples.

Response: We agree with your comment. We removed the comparison between the diversity of bacterial and fungal exometabolites, and added comparisons of exometabolite diversity among different bacterial and fungal species. Figure S4 was also modified accordingly.

Page 9, Line 268 – 274: “For bacteria, the molecular diversity of exometabolites varied considerably among species. The molecular Shannon diversity index of Gram-positive bacteria was 5.64, slightly lower than that of Gram-negative bacteria (6.19). Among the six bacterial strains, *Pantoea vagans*, *Streptomyces pratensis*, and *Stenotrophomonas* sp. showed higher molecular diversity in their exometabolites (Fig. S4a). For fungi, *Rhodotorula mucilaginosa* and *Aspergillus* species demonstrated higher molecular diversity of exometabolites (Fig. S4b). The two *Penicillium* species had similar molecular diversity, but the Chao1 index varied considerably. These results indicate the potential metabolic diversity of atmospheric bacteria and fungi.”

R3 Comment 8: Line 325, it is advised to omit the comparison between bacteria and fungi. section 3.4 “**Metabolic processes of typical isolated bacterial and fungal strains**”, the discussion of metabolic processes in bacterial and fungal in 3.4.1 and 3.4.2 is separate. In fact, in the atmosphere, the metabolism of bacteria and fungi is closely related. The authors can add a discussion of correlations between bacterial and fungal metabolic processes, thus the influence of the metabolic process of bacteria and fungi

on atmospheric environment and biogeochemical cycles can be further clarified.

Response: Thank you for your comments. In this exploratory study, we selected 12 representative microorganisms from an urban atmospheric environment, including 6 bacterial and 6 fungal strains. Given the relatively small sample size, the correlation results may be biased by this limitation when assessing metabolite product correlations. Therefore, we analyzed and discussed the correlation between bacterial and fungal metabolic processes, and we explain but prefer not show the detail in the manuscript. Page 13, Line 397 – 400: “The metabolic processes of bacteria and fungi in the surface Earth system are closely linked, which is overlooked in this study, and further elucidation of these connections will enhance our understanding of the impact of microorganisms on atmospheric environments and biogeochemical processes.” was added.

The specific results are as follows:

There were 200,716 correlations between bacterial and fungal metabolites with a correlation coefficient $|r| \geq 0.8$ and $p < 0.01$. The highest proportion of correlations (66.2%) were among bacteria-unique products, followed by those among bacteria-unique products and shared products of bacteria and fungi (20.8%) (Fig. R3a). This finding suggests that bacterial metabolites exhibited a high diversity and strong interconnections among metabolites from different bacteria. Within bacteria-unique products, only positive correlations existed, and the same for fungi-unique products. While, there were only negative correlations among bacteria-unique and fungi-unique products (Fig. R3b).

The percentage of correlations among shared products of bacteria and fungi was lower compared to the correlations among bacteria and fungi shared products and bacteria-unique products, both of which were dominated by positive correlations (Fig. R3b). Bacterial metabolites are essential in the interconnections of dissolved organic matter. The correlation network diagram shows that the bacteria and fungi shared products connected bacteria-unique and fungi-unique products, with most correlations being positive (Fig. R3c). Negative correlations were concentrated in a few shared

molecules, such as $C_{13}H_{10}O_6$, $C_{14}H_{12}O_6$, and $C_{16}H_{16}O_6$ (Fig. R3c). These shared products may serve as intermediaries between bacterial and fungal metabolites.

While many studies have explored the link between bacterial communities and dissolved organic matter, the role of fungi in organic matter transformation has often been overlooked. The results of this study offer valuable to support research on microbial contributions to the molecular composition of organic matter and interactions between organic molecules in nature ecosystems.

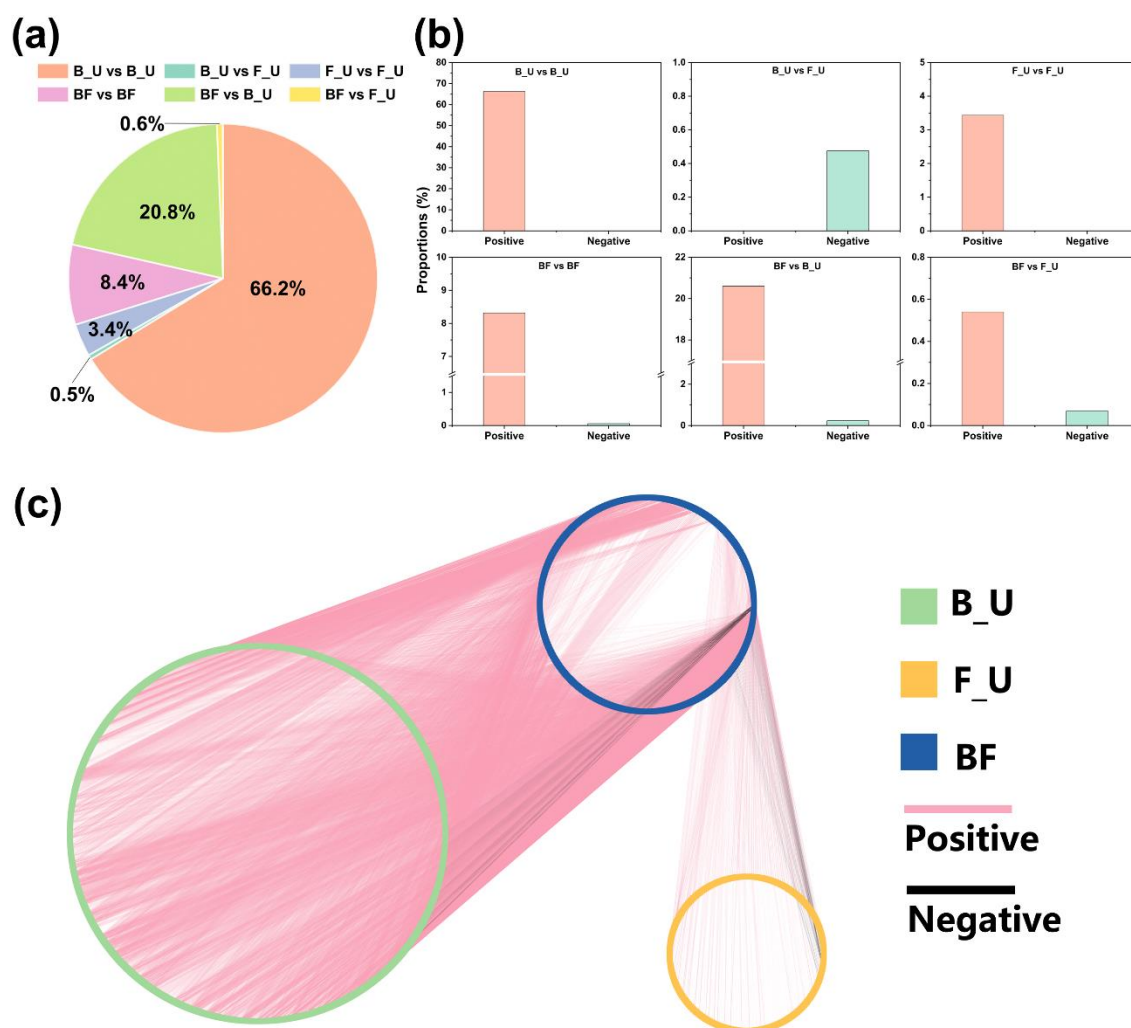


Figure R3 Correlation between bacterial and fungal metabolites. Molecules that occur only in bacterial metabolites are labeled as B_U, molecules that occur only in fungal metabolites are labeled as F_U, and molecules that occur in both bacterial and fungal metabolites are labeled as BF. (a) The pie charts characterize the overall distribution of results for metabolite correlations (Spearman's correlation coefficient $|r| \geq 0.8$, $p < 0.01$), for example, B_U vs F_U means the subgroup that shows the total number fraction of significant correlations between one bacterial-unique product and one fungal-unique

product. (b) The bar charts show the percentage of positive and negative correlations in different groups. (c) The network diagram shows the link between products shared by bacteria and fungi (BF) and products unique to bacteria (B_U) or fungi (F_U).

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Exometabolomic exploration of culturable airborne microorganisms from an urban atmosphere

Rui Jin¹, Wei Hu^{1, 2*}, Peimin Duan¹, Ming Sheng¹, Dandan Liu¹, Ziyi Huang¹, Mutong Niu¹, Libin Wu¹, Junjun Deng¹, Pingqing Fu^{1, 3*}

¹Institute of Surface-Earth System Science, School of Earth System Science, Tianjin University, Tianjin 300072, China

²Tianjin Bohai Rim Coastal Earth Critical Zone National Observation and Research Station, Tianjin University, Tianjin 300072, China

³Tianjin Key Laboratory of Earth Critical Zone Science and Sustainable Development in Bohai Rim, Tianjin University, Tianjin 300072, China

Correspondence to: Wei Hu (huwei@tju.edu.cn); Pingqing Fu (fupingqing@tju.edu.cn).

Abstract. The interactions of metabolically active atmospheric microorganisms with cloud organic matter can alter the atmospheric carbon cycle. Upon deposition, atmospheric microorganisms can influence microbial communities in surface Earth systems. However, the metabolic activities of cultivable atmospheric microorganisms in settled habitats remain less understood. Here, we cultured typical bacterial and fungal species isolated from the urban atmosphere using tryptic soy broth (TSB) and Sabouraud dextrose broth (SDB), respectively, and investigated their exometabolites to elucidate their potential roles in biogeochemical cycles. Molecular compositions of exometabolites were analyzed using ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry. Annotation through the Kyoto Encyclopedia of Genes and Genomes database helped identify metabolic processes. Results showed that bacterial and fungal strains produced exometabolites with lower H/C and higher O/C ratios compared to both consumed and resistant compounds. As CHON compounds are abundant in both TSB (85%) and SDB (78%), CHON compounds also constituted over 50% of the identified exometabolite formulas. Bacterial strains produced more abundant CHONS compounds (25.2%), while fungal exometabolites were rich in CHO compounds (31.7%). These microbial exometabolites predominantly comprised aliphatic/peptide-like and carboxyl-rich alicyclic molecule (CRAM)-like compounds. Significant variations in metabolites were observed among different microbial strains. Bacteria exhibited proficiency in amino acid synthesis, while fungi were actively involved in amino acid metabolism, transcription, and expression processes. Lipid metabolism, amino acid metabolism, and carbohydrate metabolism varied widely among bacterial strains, while fungi exhibited notable differences in carbohydrate metabolism and secondary metabolism. This study provides new insights into the transformation and potential oxidative capacity of atmospheric microorganisms concerning organic matter at air-land/water interfaces. These findings are pivotal for assessing the biogeochemical impacts of atmospheric microorganisms following their deposition.

1 Introduction

Bioaerosols include microorganisms (e.g., viruses, bacteria, fungi, archaea), various propagules (e.g., spores and pollen), biological debris, and biological metabolites (Després et al., 2012; Zhai et al., 2018). They are important components of aerosols involved in various ecological and atmospheric processes, such as climate change, hydrological, and biogeochemical cycles (Xie et al., 2021b; Kanakidou et al., 2018; Morris et al., 2011; Fröhlich-Nowoisky et al., 2016; Smets et al., 2016). Atmospheric microorganisms are usually freely floating or attached to the surface of particulate matter and can be transported over long distances by wind (Hu et al., 2020; Ruiz-Gil et al., 2020). Bacteria are emitted into the atmosphere from Earth's surfaces through aerosolization. Due to the harsh environmental stress in the atmosphere, some bacteria develop stress resistance to survive and degrade low molecular weight organic matter (Šantl-Temkiv et al., 2022; Joly et al., 2015; Ariya and Amyot, 2004). This resilience gives atmospheric microorganisms a potential role in atmospheric chemistry and physics, driving chemical reactions at environmental interfaces (e.g., the land-air interface).

Atmospheric bacteria and fungi can maintain metabolic activities due to specific growth characteristics, such as spore production capacity, ultraviolet resistance, drought resistance, or through extracellular secretions (Matulová et al., 2014; Huang and Hull, 2017; Bryan et al., 2019). For example, *Aspergillus niger* resists ultraviolet damage with pigmented spores, and *Sphingomonas aerolata* NW12 maintains cellular functions by increasing rRNA content and producing proteins while aloft (Cortesao et al., 2020; Krumins et al., 2014). Atmospheric microbial activity is affected by freeze-thaw and condensation/evaporation cycles, while microbially produced extracellular polymeric substances (composed primarily of polysaccharides, proteins, and nucleic acids) help protect cells from osmotic stress (Matulová et al., 2014; Joly et al., 2015). Additionally, a large number of active microorganisms in air and cloud water are involved in atmospheric chemical processes. Atmospheric microorganisms play a dual role in atmospheric chemistry and biogeochemical cycles. **Not only do they directly metabolize organic carbon, but they can also reduce the availability of radical sources, thereby decreasing the oxidative capacity of cloud systems (Zhang et al., 2019; Lallement et al., 2018a; Vaitilingom et al., 2013).** Early studies revealed that some bacteria isolated from cloud water could degrade small organic molecules (e.g., phenol, formaldehyde, carboxylic acids, and methanol), thereby influencing the organic carbon budget and oxidative balance in clouds (Vaitilingom et al., 2010; Amato et al., 2007; Vaitilingom et al., 2011; Lallement et al., 2018b; Jaber et al., 2020). For example, *Rhodococcus enclensis*, a highly active species isolated from clouds, biodegrades catechol significantly faster than phenol (Jaber et al., 2020). Metatranscriptomic analysis and laboratory incubation experiments showed that 93% of 145 bacterial strains isolated from clouds over Puy de Dôme could degrade phenol (Lallement et al., 2018b). Furthermore, laboratory experiments indicate that environmental factors such as radiation and pH can influence the biodegradation of organic acids by atmospheric bacteria (Liu et al., 2023b).

Atmospheric microorganisms deposited through wet and dry deposition are a crucial biological source in ocean and freshwater biogeochemical cycles, particularly in oligotrophic systems. Numerous studies highlight the significant role of atmospheric deposition in influencing the nitrogen cycle in marine environments. Mesocosm experiments have demonstrated that the addition of Saharan dust led to a rapid (within 30 hours), pronounced (2–4 fold), and sustained (over 6 days) enhancement in N₂ fixation rates, which contributed 3–8% to primary productivity (Rahav et al., 2016b). Aerosol deposition, especially dust, can deliver active bacteria to the ocean surface and support viable microorganisms like diazotrophs, enhancing N₂ fixation and altering marine bacterial communities (Rahav et al., 2016a; Rahav et al., 2018; Na et al., 2023). When airborne microorganisms settle in high-nutrient waters, the dormant spores of autotrophs germinate under favorable conditions and rapidly increase (Genitsaris et al., 2021). These results reveal the potential biogeochemical impacts of the atmospheric deposition of microbes carried by aerosols in surface water.

Wet deposition (e.g., rainfall) brings a wealth of nutrients and microorganisms to surface waters, increasing chlorophyll *a* concentration and enhancing carbon and nitrogen fixation. Short-term wet deposition events play an important role in the temporal variation of new nitrogen production and phytoplankton dynamics in the southeastern Mediterranean, as demonstrated by both experimental and in situ observations (Rahav et al., 2021). Additionally, one notable study demonstrated that rainwater influenced by Saharan dust significantly increased bacterial populations in high-altitude lake water, with rare taxa becoming dominant based on cultivation experiments (Peter et al., 2014). Despite these findings, there is a lack of research on how atmospheric microorganisms metabolize and degrade organic matter, making it challenging to accurately assess their metabolic processes and potential impacts on atmospheric chemistry and biogeochemical cycles. Therefore, further research is needed to explore the metabolome of atmospheric microorganisms to understand better the essential processes that influence the Earth's atmosphere and ecosystems.

Metabolomics provides direct insights into the function within an ecosystem (Bauermeister et al., 2021). For example, marine phytoplankton generate large amounts of exometabolites, forming a carbon pool known as extracellular release or dissolved primary production (Moran et al., 2022). Microbial exometabolites hold great ecological significance, as they can serve as substrates for other microorganisms, facilitating population growth (Douglas, 2020). For instance, the metabolome of *Pseudomonas graminis* exposed to oxidative stress involves carbohydrate, glutathione, energy, lipid, peptide, and amino acid metabolism pathways (Wirgot et al., 2019). Furthermore, microbial exometabolites, including extracellular polymeric substances and pigments, are vital for the survival of atmospheric microorganisms, enabling them to withstand long-range transport and extreme conditions in the atmosphere or at the ocean surface (Erkorkmaz et al., 2023; Bryan et al., 2019). Recently, a framework for atmo-ecometabolomics, from sampling to data analysis, was developed to characterize the molecular composition of aerosols (Rivas-Ubach et al., 2019). This framework also provides insights into how aerosol chemical compositions impact ecosystem structure, function, and biogeochemistry. However, previous studies have not fully explored the role of metabolically active microorganisms in atmospheric aerosols. Deepening our understanding of the metabolome of atmospheric microorganisms is essential for predicting their ecological roles in biogeochemical cycles and assessing their broader impacts on ecosystem functions and atmospheric processes.

Mass spectrometers with different mass analyzers, including time-of-flight, Orbitrap, and FT-ICR, are favored in many metabolomics applications (Bauermeister et al., 2021). FT-ICR MS is a tool for obtaining high-resolution metabolomic fingerprints and a deeper understanding of potential chemical transformations in various types of samples, such as landfill leachate, algal products, seawater, lake water, soil, rainwater and aerosols (Yuan et al., 2017; Gonsior et al., 2019; Bahureksa et al., 2021; Xie et al., 2021a; Qi et al., 2022). Relevant biosynthetic pathways can be traced through chemical information, relying on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Lopez-Ibañez et al., 2021). KEGG is a comprehensive database resource designed to facilitate interpreting high-level functions and systems by integrating molecular-level data, including cellular, organismal, and ecological processes. This database is particularly valuable for analyzing large-scale molecular datasets derived from genomic sequencing and other high-throughput technologies, enabling the exploration of complex biological pathways and networks (Kanehisa et al., 2017). Biochemical pathways were identified by mapping the molecular composition of metabolites obtained by FT-ICR MS to chemical structures in the KEGG COMPOUND Database using the analytical pipeline developed by Ayala-Ortiz et al. (2023). Previously, organic compounds in the mass range of ~80–1000 Da have generally been unidentified and overlooked in aerosol particles, yet they may play crucial roles in the ecosystem functioning, especially at the atmosphere-biosphere interface.

This study performed a non-targeted screening of microbial exometabolomes generated during the growth of atmospheric culturable microorganisms, using FT-ICR MS coupled to negative ion mode electrospray ionization (ESI[−]). The aim was to reveal the characteristics of metabolites from active atmospheric microbes and to underline the important connection between atmospheric microbes and subsurface environments. The specific objectives of this study were to (i) explore the molecular characteristics of exometabolites produced by typical atmospheric culturable bacterial and fungal strains, (ii) elucidate the central metabolic processes and the differences among different strains, and (iii) unravel the potential metabolic capacities of these typical bacteria and fungi. This work provides data to support the roles of atmospheric microorganisms in affecting atmospheric organic matter and biogeochemical processes after deposition onto oceanic or freshwater surfaces.

2 Materials and methods

2.1 Samples collection and microbial isolation

The sampling site was located on the roof of Building No.19 on the Weijin Road campus of Tianjin University (39.11°N, 117.16°E, about 21 m above the ground level) in an urban area (Fig. S1a) about 500 m from the main road. Total suspended particulate matter (TSP) samples were collected onto quartz fiber filters (10 in. × 8 in.) at a flow rate of 1.05 m³ min^{−1} from 15:00 to 19:00 on 5th, 8th, and 11th January 2022. All samples were stored at −20°C until subsequent treatment. Before sampling, quartz fiber filters were heated in a muffle furnace at 450°C for 6 h to remove organic matter.

Tryptic soy agar (TSA) and Sabouraud dextrose agar (SDA) media were used for bacterial and fungal isolation, respectively. The compositions of the media are detailed in Table S1. All media were sterilized in an autoclave at 115°C for 20 min, and

then solid culture plates were prepared under aseptic conditions. Under sterile conditions, one-eighth of each filter sample was cut and placed in a 50 mL centrifuge tube with 40 mL of 1×phosphate buffer saline (PBS) solution. Each sample underwent three parallel experiments. Microorganisms on the filters were detached using low-power ultrasonication in an ice bath for 5 min and then centrifuged at 250 rpm for 30 min. Then, a 100 µL aliquot of the well-mixed suspension was spread-plated onto the two types of solid media. For each suspension, three independent replicates were prepared to ensure that both media were thoroughly coated and evaluated. The plates were incubated at a constant temperature of 37°C for 48 h for bacteria, and at 28°C for 72 h for fungi, with daily growth monitoring (Wang et al., 2023; Timm et al., 2020). From each plate, all phenotypically distinct colonies were picked up and smeared onto fresh media for isolation. Single colonies were picked and re-streaked from each plate at least three times to isolate individual strains (Timm et al., 2020). Multiple streaking isolations are essential to obtain stable and pure strains by gradually diluting the microbial population and allowing for the isolation of individual colonies (Yan et al., 2021; Dunbar et al., 2015).

Isolated strains were preserved using the cryopreservation method. Bacterial strains were cultured in tryptic soy broth (TSB) at 37°C with shaking at 200 rpm, while fungal strains were cultured at 28°C on SDA medium plates under a stationary condition. When the bacteria reached the logarithmic phase, 750 µL of the culture was mixed thoroughly with an equal volume of 50% glycerol, resulting in a final glycerol concentration of 25%, and then stored at –80°C. For fungal strains, once spores were evenly distributed across the plate, they were eluted with 4 mL of sterile PBS buffer. A 750 µL aliquot of the spore suspension was then mixed with an equal volume of 50% glycerol and stored at –80°C with a final glycerol concentration of 25%.

2.2 DNA extraction and microbial identification

Liquid cultures were inoculated with single colonies and grown to the logarithmic phase. DNA was extracted from 2 mL liquid cultures using the Universal Genomic DNA Extraction Kit (Solarbio, Beijing, China). The DNA was then stored at –80°C. The 16S rRNA gene region was amplified using 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') primers. The PCR reaction volume was 25 µL, containing 1 µL genomic DNA, 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 13 µL 2×Taq PCR Mix (Vazyme, Nanjing, China), and 9 µL ddH₂O. The PCR program was: initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, extension at 72°C for 1 min; and a final extension at 72°C for 10 min. The fungal internal transcribed spacer (ITS) regions were amplified by PCR (94°C for 5 min; followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min; final extension at 72°C for 10 min) using the ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers. The PCR reaction system for fungi was the same as that for bacteria.

The PCR products were checked by 1% agarose gel electrophoresis and then purified by Universal DNA Purification Kit (TIANGEN, Beijing, China). The sequences of the purified gene fragments were determined by Sanger sequencing (BGI Genomics Co., Ltd, Beijing, China). Taxonomic assignments were determined from 16S rRNA gene sequences or ITS sequences using the BLAST program at NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

160 2.3 Microbial growth conditions and metabolite collection

Six representative bacterial strains and six typical fungal strains were selected from the isolated atmospheric microorganisms for subsequent exometabolome studies. Single colonies of all bacterial strains were picked and inoculated in 50 mL TSB medium and incubated at 37°C, 200 rpm for 12 h to obtain the primary seed solution. The primary seed solution was transferred to a 100 mL flask containing 50 mL of sterilized liquid medium at an inoculum level of 3 mL (6%) and incubated for 12 h at 165 37°C with shaking to obtain the secondary seed solution. The secondary seed solution was also transferred to 50 mL sterilized liquid medium at an inoculum level of 3 mL (6%) and incubated for 7 days at 37°C, 200 rpm. Cultures were then centrifuged at 4500 rpm for 20 min at 4°C. The metabolic product supernatant of about 40 mL was transferred into a new centrifuge tube and stored at –20°C until analysis of molecular composition with FT-ICR MS.

For all fungi strains, spore suspensions were prepared with sterile PBS buffer by eluting spores from fungal plates incubated 170 at 28°C for 7 days. The spore suspension concentration was calculated using a hemocytometer. Then, about 10⁸ cells were inoculated into 50 mL Sabouraud dextrose broth (SDB) and incubated for 15 days at 200 rpm in a 28°C shaker. The method of obtaining and preserving the metabolic products was identical to that used for bacterial cultures.

2.4 FT-ICR MS analysis

The metabolic products (2 mL) filtered by 0.22 µm pore membranes were acidified to pH 2 using high-pressure liquid 175 chromatography (HPLC) grade hydrochloric acid (HCl). Dissolved organic matter (DOM) was extracted using a solid phase extraction (SPE) cartridge (200 mg, Oasis HLB, 6cc, Waters, U.S.) to remove salts (Chen et al., 2022; Han et al., 2022). After extraction, the cartridges were dried by flushing with high-purity N₂. Finally, 6 mL of HPLC-grade methanol (Sigma-Aldrich) was used to elute the extracted DOM. A 2 mL aliquot of the eluent was collected and stored at –20°C for further analysis. The eluent was analyzed with a 7.0 T superconducting magnet Bruker Solarix FT-ICR MS (Solarix 2xR, Bruker, Germany) 180 equipped with an electrospray ionization (ESI) source in the negative ion mode. The samples were directly injected into the device at a continuous flow rate of 150 µL h^{–1} with a capillary voltage of 5000 V and an ion accumulation time of 0.028 s. The signal acquisition process included 256 cumulative scans and a 4M transient to obtain a higher signal-to-noise ratio (S/N) to resolve the sample fully. More detailed instrument parameter information is available in previous studies (Su et al., 2021). Since FT-ICR MS determines potent polarity molecules with molecular weight (MW) from 100 Da to 1000 Da, pigments or 185 extracellular enzymes secreted by bacteria or fungi can be detected and identified. The complete experimental procedure is shown in Fig. S1b.

2.5 Data processing and statistical analysis

The raw datasets were calibrated using the DataAnalysis (ver. 5.0, Bruker Daltonics). The elemental compositions for all recalibrated peaks with S/N ≥ 4, using a mass allowance of ±1.0 ppm, were assigned using Composer (Sierra Analytics, USA) 190 software. The molecular formulas were screened according to the criteria: C₀₋₅₀H₀₋₁₀₀O₀₋₅₀N₀₋₁₀S₀₋₃P₀₋₃, 0.3 ≤ H/C < 2.5, O/C <

1.2, N/C < 0.5, S/C < 0.2 (Chen et al., 2021b; Yu et al., 2019). Based on the H/C and O/C ratios of the molecular formulas, all formulas were classified into seven categories: lipid-like ($1.5 < \text{H/C} \leq 2.0$, $0 \leq \text{O/C} \leq 0.3$), aliphatic/peptide-like ($1.5 < \text{H/C} \leq 2.2$, $0.3 < \text{O/C} \leq 0.67$), carbohydrate-like ($1.5 < \text{H/C} \leq 2.5$, $0.67 < \text{O/C} < 1.0$), unsaturated hydrocarbons ($0.67 < \text{H/C} \leq 1.5$, $\text{O/C} < 0.1$), carboxyl-rich alicyclic molecule (CRAM)-like ($0.67 < \text{H/C} \leq 1.5$, $0.1 \leq \text{O/C} < 0.67$), aromatic-like ($0.2 \leq \text{H/C} \leq 0.67$, $\text{O/C} < 0.67$), and tannin-like/highly oxygenated compounds (HOC) ($0.6 < \text{H/C} \leq 1.5$, $0.67 \leq \text{O/C} \leq 1.0$) (Bianco et al., 2018).

Depending on the elemental composition of the compound, the molecular formulas were divided into CHO, CHON, CHOS, CHONS, and CHONSP compounds. For example, CHO indicates that the organic compounds in this category contain only three elements: carbon, hydrogen, and oxygen, with no other elements. The potential metabolic processes of microorganisms were annotated at a molecular level and analyzed using the MetaboDirect pipeline, and the annotation of metabolic pathways mainly relied on the KEGG database (Ayala-Ortiz et al., 2023; Ogata et al., 1999). The molecular mass-based transformation was analyzed by calculating the differences between a pair of m/z for all peaks in each sample and comparing them to the list of pre-defined masses of common metabolic reactions (biochemical transformations key). The transformation networks encompass processes related to pigment molecules, which are visualized using Cytoscape version 3.10.2. The KEGG database contains three levels of metabolic pathways: 7 classes of primary pathways, 59 classes of secondary pathways, and 563 classes of tertiary pathways (Kanehisa et al., 2017). The Shannon and Chao1 index were calculated in R using the “vegan” (v2.6–4) packages. All statistical analyses were performed in R Studio for R version 4.2.1 and OriginPro 2023b.

3 Results

3.1 Composition of culturable atmospheric microorganisms

Twenty-four bacterial and sixteen fungal strains were isolated from the TSP samples (Tables S2 and S3). Fig. 1 illustrates the dominant taxa of culturable bacteria and fungi at the phylum and genus levels. The culturable bacterial community primarily consisted of *Pseudomonadota* (45.8%), *Bacillota* (37.5%), and *Actinomycetota* (16.7%). The dominant genera were *Bacillus* (37.5%), *Pseudomonas* (25%), and *Streptomyces* (16.7%). The isolated bacterial genera represent major taxa commonly found in the atmosphere (Yan et al., 2021; Lee et al., 2017; Calderon-Ezquerro et al., 2021). Gram-positive bacteria such as *Bacillus* and *Streptomyces* possess cell walls predominantly composed of N-acetylmuramic acid. While, Gram-negative bacteria such as *Pantoea*, *Erwinia*, *Stenotrophomonas*, and *Pseudomonas* have cell walls rich in lipopolysaccharides (LPS)/endotoxin (Ruiz-Gil et al., 2020).

In the culturable fungal community, Ascomycota (87.5%) was overwhelmingly dominant. The most abundant fungal genera were *Aspergillus* (37.5%) and *Penicillium* (25%), collectively accounting for over 50% of the isolated strains (Fig. 1). These findings are consistent with previous studies, which have also identified Ascomycota as the most abundant fungal phylum across different pollution levels and seasons (Abd Aziz et al., 2018; Drautz-Moses et al., 2022; Cáliz et al., 2018; Pyrri et al., 2020). To investigate the potential influence of microbial secondary metabolites in atmospheric and biogeochemical processes,

we selected representative bacterial and fungal strains for further study (Table S4 and Fig. S2). In the following sections, microbial species names are used instead of strain names for conciseness. Our primary focus was on the exometabolome of these atmospheric microorganisms, aiming to elucidate and analyze the metabolic potential and capacity to influence atmospheric biogeochemistry.

3.2 Changes of organic molecules in culture media after microbial incubation

The nutrients in the initial culture media supply the growth and reproduction of microorganisms. Through diverse metabolic processes, microorganisms consume substantial amounts of bioavailable organic matter and produce a variety of secondary metabolites. The organic molecular compositions of the media, before and after microbial incubation, were characterized using FT-ICR MS. Organic molecules were grouped into three categories: (1) those present in the initial medium but absent after incubation, indicating consumed organic matter; (2) those absent in the initial medium but present after incubation, representing biologically controlled organic matter produced by the microorganisms, define as exometabolites; (3) those present in both the initial medium and after incubation, indicating resistant organic matter (Yuan et al., 2017). Some resistant organic molecules may be partially produced by microorganisms and are not classified as exometabolites, as FT-ICR-MS data are not quantitative and cannot unequivocally confirm changes in the abundance of these compounds (Labrie et al., 2022; Noriega-Ortega et al., 2019). Due to limitations in FT-ICR MS techniques, isomers were not considered in this study.

Molecular changes during the metabolic processes of typical atmospheric bacteria (Fig. 2) and fungi (Fig. 3) are demonstrated using van Krevelen diagrams and stacked bar charts, with the formula numbers provided in Table S5. In the bacterial initial medium (TSB), a total of 3416 formulas were detected, with CHON compounds dominating both in number (85.3%) and intensity (92.7%) (Fig. S3a). Furthermore, lipid-like (13%), aliphatic/peptide-like (34%), and CRAM-like (52%) compounds collectively represent 99% of the total number of formulas (Fig. S3b). The bacterial products had a lower H/C ratio (mean \pm SE = 1.43 ± 0.05) and higher O/C ratio (0.46 ± 0.01) (Fig. 2a). They are more oxidized than consumed (H/C = 1.57 ± 0.01 ; O/C = 0.32 ± 0.01) and resistant molecules (H/C = 1.46 ± 0.00 ; O/C = 0.39 ± 0.00). These bacteria consumed 413–1198 formulas, primarily in the categories of lipid-like (19–37%), aliphatic/peptide-like (27–43%), and CRAM-like (31–44%) compounds (Fig. 2b). *Stenotrophomonas* sp. and *Pseudomonas baetica* consumed more aliphatic/peptide-like and CRAM-like compounds (in terms of formula number) than other strain. CRAM-like compounds dominated (54–60%) resistant organic matter. All bacterial strains produced organic molecules containing predominantly CRAM-like compounds (51–64%) except for *P. baetica* (38%).

In the fungal initial medium (SDB), 3920 formulas were detected, with CHON compounds dominating both in number (77.9%) and intensity (89.4%) (Fig. S3c). Furthermore, lipid-like (14%), aliphatic/peptide-like (32%), and CRAM-like (51%) compounds collectively represent 97% of all molecules in terms of number (Fig. S3d). The formula elemental composition and category of fungal initial medium is similar to that of bacterial initial medium. The molecules consumed by the fungi were mostly compounds with low O/C ratios (0.34 ± 0.01) and high H/C ratios (1.51 ± 0.02) (Fig. 3a). The fungal products had elevated O/C ratio (0.44 ± 0.02) and reduced H/C ratio (1.32 ± 0.06), which also suggests that airborne microorganisms have

the potential to influence atmospheric oxidative capacity and organic carbon budget. The six typical fungi consumed 811–2531 formulas during incubation, with key categories including lipid-like (16–34%), aliphatic/peptide-like (25–32%), and CRAM-like (32–53%) compounds (Fig. 3b). Unlike bacteria, fungi almost exclusively consumed unsaturated hydrocarbons for their growth, with no unsaturated hydrocarbons remaining in the resistant molecules or products. Notably, *Talaromyces* sp. consumed 2531 formulas, more than any other fungal strains, indicating a higher diversity of consumed compounds compared to resistant (1389 formulas) and produced (246 formulas) compounds (Table S5). The resistant compounds for fungal strains were rich in aliphatic/peptide-like (31–37%) and CRAM-like (50–61%) compounds. The product composition of *A. niger* was significantly different, with 20% lipid-like molecules, much larger than the other fungi.

3.3 Molecular characteristics of exometabolites from typical cultivable bacteria and fungi

3.3.1 Molecular diversity of bacterial and fungal exometabolites

As mentioned above, 651–2868 formulas were produced by the bacterial strains, and 246–1501 formulas were produced by the fungal strains (Table S5), providing a basis for exometabolomic analysis. The molecular diversity of bacterial and fungal exometabolites is illustrated in Fig. S4. For bacteria, the molecular diversity of exometabolites varied considerably among species. The molecular Shannon diversity index of Gram-positive bacteria was 5.64, slightly lower than that of Gram-negative bacteria (6.19). Among the six bacterial strains, *Pantoea vagans*, *Streptomyces pratensis*, and *Stenotrophomonas* sp. showed higher molecular diversity in their exometabolites (Fig. S4a). For fungi, *Rhodotorula mucilaginosa* and *Aspergillus* species demonstrated higher molecular diversity of exometabolites (Fig. S4b). The two *Penicillium* species had similar molecular diversity, but the Chao1 index varied considerably. These results indicate the potential metabolic diversity of atmospheric bacteria and fungi.

3.3.2 Molecular composition of exometabolites from typical bacterial strains

There are significant differences in the molecular characteristics of exometabolites among the bacterial strains (Fig. 4). The spectral peaks of the exometabolites displayed an uneven distribution across different bacterial strains (Fig. 4a). Overall, CHON compounds accounted for over 50% of the total formula numbers, followed by CHONS compounds, which had an average proportion of 25.2% (Fig. 4a). Given the high abundance of CHON compounds in the initial culture media, CHON compounds were also the primary constituents of the high abundance categories of bacterial exometabolites (Fig. S5a). Moreover, a large number of fractions (18.1–40.6%) of CHONS compounds were produced by the bacterial strains (Fig. 4a and Table S7), much higher than in the initial medium (4.2%). The bacterial exometabolites predominantly consisted of lipid-like, aliphatic/peptide-like, CRAM-like, and tannin-like/highly oxygenated compounds (Fig. 4b). Significant variations were observed in the exometabolomic profiles of different bacterial species. Notably, *P. vagans* produced a higher proportion of nitrogenous unsaturated molecules (e.g., $C_{12}H_{10}N_2O$, $C_{15}H_{12}N_2O$, $C_{13}H_{12}N_2O$). *Stenotrophomonas* sp. and *P. baetica*, both Gram-negative bacteria, metabolized and synthesized large quantities of CHONS compounds (55.3–61.8%

of intensity), largely differed from the other four bacteria strains (only 3.5–21.5% of intensity). Notably, in exometabolites from *Stenotrophomonas* sp., CHONS compounds dominated the lipid-like and aliphatic/peptide-like molecules, distinguishing it from the other bacterial strains (Fig. 4b). Microbes can convert bioavailable lipid-like and protein-like nitrogenous compounds into more oxygenated, unsaturated (more refractory) nitrogenous CRAM/lignin-like compounds (Osborne et al., 2013). Additionally, *Stenotrophomonas* sp. produced a higher number (903 formulas) of high molecular weight compounds ($m/z > 500$) (Table S6), likely representing extracellular polymeric substances, e.g., polysaccharides, extracellular enzymes, and cellular debris (Vandana et al., 2023; Moradali and Rehm, 2020).

Comparing the unique molecules in the exometabolites of different strains provides insights into their distinct metabolic processes, and helps to understand the impacts of atmospheric microbial deposition on microbial metabolic activity and the biogeochemical cycle in aquatic ecosystems. A total of 253 common formulas existed among the six typical bacteria, predominantly CHON compounds concentrated in aliphatic/peptide-like and CRAM-like compounds (Fig. 5a and S5b). The unique formulas of each bacterial species contained CHON compounds, with proportions exceeding 25% (Fig. 5a). Most CHON metabolites had an O/N ratio ≤ 3 , suggesting they may be associated with amino and amide groups or N-heterocyclic-containing combinations (Fig. 4c). The metabolism of *P. vagans* appeared to yield unsaturated hydrocarbons compounds containing amino or amide groups, based on molecular classification. *Stenotrophomonas* sp. had the highest percentage (36%) of unique formulas, indicating more diverse metabolic processes (Fig. 5a). Certain *Stenotrophomonas* species can produce polyamines, indole-3-acetic acid ($C_{10}H_9NO_2$), and cytokinins (e.g., $C_{10}H_9N_5O$, $C_9H_8N_4OS$, $C_{17}H_{19}N_5O_5$), which contribute to their ability to protect plants (Peleg and Abbott, 2015; Zhao et al., 2024).

For Gram-positive bacteria, the elemental compositions of the unique formulas were similar, with CHON compounds accounting for more than 50% of all compounds (Fig. 5a). In contrast, for Gram-negative bacteria, CHON compounds were predominant (48.1%) in the unique formulas specific to *P. vagans*, and CHONS compounds are predominantly those specific to *Stenotrophomonas* sp. and *P. baetica*, with proportions of 70.2% and 71.4%, respectively (Fig. 5a). In addition, CHO compounds comprised over 25% of the unique formulas found exclusively in *P. vagans* (Fig. 5a). Notably, among these unique CHO compounds, the most abundant formulas belonged to the O_9 class, dominated by $C_{18}H_{28}O_9$ and $C_{13}H_{16}O_9$, indicating higher oxidation states (Fig. S5c). The oxygen numbers of unique CHONS compounds from *Stenotrophomonas* sp. were mainly concentrated in the range of O_7 to O_{11} (Fig. S5d), suggesting a higher level of oxidation, probably due to the presence of $-OSO_3H$, $-ONO_2$, or other oxygen-containing groups such as hydroxyl or carboxyl groups. All CHONS compounds specific to *P. baetica* were CHO_nNS (Fig. S5e), with most being $CHO_{10-13}NS$ compounds. These results suggest that these highly oxidized compounds could act as indicator molecules for differentiating among different bacterial species.

3.3.3 Molecular composition of exometabolites from typical fungal strains

For fungal exometabolites, CHON compounds also dominated, comprising over 50% of the total formula number on average, followed by CHO compounds (31.7%) (Fig. 6a). The number of formulas identified in fungal exometabolites was lower compared to bacteria (Table S6). Unlike the bacterial strains, which all produced high fractions of CHONS compounds,

320 CHONS compounds appeared only in exometabolites of *R. mucilaginosa*, a yeast species, accounting for approximately 19% of the total formula number (Fig. 6a). This indicates a diversity of sulfur-related metabolic processes for *Rhodotorula*. In contrast, the exometabolites produced by mold fungi, specifically from the genera *Penicillium* (*Penicillium oxalicum* and *Penicillium aurantiogriseum*) and *Talaromyces* (*Talaromyces* sp.), were predominantly CHO compounds, accounting for over 50% of the total exometabolite intensity (Fig. 6a). Among these CHO compounds produced by *Penicillium* and *Talaromyces*, 325 the formulas with the highest intensity were $C_{12}H_{16}O_6$, $C_{15}H_{10}O_6$, and $C_{15}H_8O_7$. The exometabolites from *Talaromyces* sp. were even more specific, with CHO compounds representing 98% of the total intensity and 74% of the formula number. Fungal exometabolites were mainly composed of aliphatic/peptide-like, and CRAM-like compounds (Fig. 6b). *Talaromyces* sp. produced aliphatic/peptide-like molecules consisting exclusively of CHON compounds, and CRAM-like compounds containing mainly CHO compounds (Fig. S6a). The highest-intensity compounds of *Talaromyces* sp., $C_{15}H_8O_7$ and $C_{16}H_{16}O_5$, 330 belong to aromatic-like and CRAM-like compounds, respectively. *Talaromyces* can produce many bioactive secondary metabolites, with $C_{15}H_8O_7$ and $C_{15}H_{10}O_6$ identified as emodic acids and catenarin, respectively (Lei et al., 2022). These were identified in this study as the leading exometabolites of *Talaromyces* sp, and they are also important products of *Talaromyces avellaneus* and *Talaromyces stipitatus* (Zhai et al., 2016). Among the exometabolites of the six selected fungi, only 12 formulas were common, including eight CHO compounds and 335 four CHON compounds (Fig. 5b). This is considerably fewer compared to the common formulas observed in bacteria, highlighting the tremendous difference in fungal exometabolites. There were also significant variations in the unique molecule compositions of exometabolites from different fungi. As mentioned above, *R. mucilaginosa* exhibited distinctive molecules characterized by a substantial presence of CHONS compounds (Fig. 5b). A relatively high percentage of these CHONS compounds possess an oxygen number greater than five (Fig. S6b), indicating a high degree of oxidation, with possible 340 functional groups such as amino, amide, and sulfonyl groups (Liu et al., 2021a; Song et al., 2018). Most of the unique formulas in the exometabolites of the six typical fungi were CHON compounds with an O/N ratio ≤ 6 , mainly in aliphatic/peptide-like and CRAM-like classes (Fig. 6c), with the possible presence of amino, amide, and nitro function groups. Significantly different from the bacteria, the genera of *Penicillium* and *Talaromyces* generated very distinctive pigmentation during cultivation, with some of these pigment molecules classified as CRAM-like compounds (Fig. S7). Compared to the molecular formulas of 345 pigments resolved in previous studies (Morales-Oyervides et al., 2020; Contreras-Machuca et al., 2022), 10 and 8 typical pigment molecules were identified in the exometabolites from *Penicillium* and *Talaromyces*, respectively (Table S8).

3.4 Metabolic processes of typical isolated bacterial and fungal strains

3.4.1 Biochemical transformation of bacterial and fungal strains

Microorganisms can selectively uptake substrates and undergo diverse metabolic transformations. During bacterial and fungal 350 growth, there were 168924 and 70646 potential molecular transformations, respectively, with 139798 (83%) transformations unique to bacteria and 41520 (59%) to fungi (Fig. S8a). Among the bacterial transformations, *Stenotrophomonas* sp. had 51667

unique transformations, which accounted for 58% of the total for this strain, demonstrating a higher diversity of metabolite interconversions (Fig. S8b). *P. aurantiogriseum* showed the highest number (24702) of unique transformations, accounting for 65% of its total number of transformations. In contrast, *P. oxalicum* had fewer unique transformations, with 3867 (32%) transformations (Fig. S8c). These results suggest that the potential metabolic pathways vary greatly among *Penicillium* species, highlighting the importance of further studies to characterize the metabolism across different *Penicillium* species.

According to the classification rules of Ayala-Ortiz et al. (2023), 95 and 99 transformation types were identified for bacterial and fungal strains, respectively. Methylation and oxidation/hydroxylation were the most prevalent potential transformations during microbial metabolism (Fig. 7a and 7b). This result is consistent with the metabolic responses of peatland microorganisms to moss leachate and *Pseudoalteromonas* to phage infections (Fudyma et al., 2021; Ayala-Ortiz et al., 2023). Amino acid metabolism differed markedly among bacteria, with alanine, isoleucine, and leucine transformation types being more prominent for *B. subtilis*, while glutamic acid transformation was more abundant for *Stenotrophomonas* sp. (Fig. 7a). For *Talaromyces* sp., carboxylation, glyoxylate, and erythrose transformations were dominant, significantly different from the other fungi (Fig. 7b).

There were significant differences in transformation numbers between bacteria and fungi, with abiotic and amino acid transformations being the most prevalent (Fig. S8d). Notably, cysteine and methionine transformations related to amino acids were significantly higher in bacteria than in fungi (Fig. S8e). In contrast, transformation types associated with phospholipid, biotin, and cytidine were more highly represented in fungi. Therefore, it is hypothesized that bacteria exhibit a stronger inclination towards amino acid synthesis, whereas fungi are more active in transcription and expression.

Transformation networks are frequently used to analyze the metabolic states of organisms since the underlying biochemical reactions are well-known (Plamper et al., 2023). The main organic molecules involved in pigment transformations were CRAM-like and aliphatic/peptide-like compounds for *P. aurantiogriseum* (Fig. 7c) and CRAM-like and aromatic-like compounds for *Talaromyces* sp. (Fig. 7d), respectively. The transformation networks showed interactions within a cluster of CRAM-like metabolites. This suggests that these fungi can efficiently convert certain peptides into stable pigment molecules, which may have industrial applications.

3.4.2 Metabolic pathways of typical isolated bacterial and fungal strains

A total of 96 and 53 KEGG metabolic pathways were annotated from typical bacterial and fungal exometabolites, respectively. For bacterial exometabolites, 16 secondary pathways were annotated, with carbohydrate metabolism, xenobiotics biodegradation and metabolism, biosynthesis of other secondary metabolites, and amino acid metabolism being the major pathways (Fig. S9a). For fungi, 12 secondary pathways were annotated, with the major pathways similar to those for bacteria (Fig. S9b), but xenobiotics biodegradation and metabolism were not annotated for fungi.

The primary KEGG Level 3 pathways varied widely among bacteria (Fig. 8a). *P. vagans* and *B. subtilis* were mainly annotated to arachidonic acid metabolism (KO00590) within lipid metabolism. *B. subtilis*, *S. pratensis*, and *Stenotrophomonas* sp. were more oriented towards carbohydrate metabolism, with the main pathways including ascorbate and aldarate metabolism

385 (KO00053), amino sugar and nucleotide sugar metabolism (KO00520), and pentose and glucuronate interconversions (KO00040). For *Stenotrophomonas* sp., amino acid metabolism of tyrosine metabolism (KO00350) and histidine metabolism (KO00340) were more enriched. Bacteria and fungi shared 16 pathways, mainly related to carbohydrate metabolism, energy metabolism, and membrane transport, which are essential metabolic processes that sustain cell growth and reproduction. The six typical fungal strains can be classified into different groups according to the KEGG Level 3 pathways (Fig. 8b). *R. mucilaginos* 390 *osa* and *Penicillium* species were mainly involved in carbohydrate metabolism, specifically fructose and mannose metabolism (KO00051) and galactose metabolism (KO00052). Additionally, they were characterized by lysine biosynthesis (KO00300), ABC transporters (KO02010), and diterpenoid biosynthesis (KO00904). *Talaromyces* sp. was predominantly involved in aflatoxin biosynthesis (KO00254), while *Aspergillus* metabolism was dominated by arginine and proline metabolism (KO00330), methane metabolism (KO00680) and cyanoamino acid metabolism (KO00460).

395 Analytical approaches that map molecular composition to microbial metabolic pathways offer a powerful framework for understanding the complex metabolic functions and key transitions in microbial processes. This helps to elucidate the unique metabolic processes by which microorganisms survive and adapt in atmospheric environments. The metabolic processes of bacteria and fungi in the surface Earth system are closely linked, which is overlooked in this study, and further elucidation of these connections will enhance our understanding of the impact of microorganisms on atmospheric environments and 400 biogeochemical processes.

4 Discussion

4.1 Survival mechanisms of culturable atmospheric microorganisms

At the genus level, culturable atmospheric bacteria isolated from the aerosol samples mainly included *Bacillus*, *Pseudomonas*, *Streptomyces*, and *Pantoea* (Fig. 1). These bacterial taxa were predominant across urban, rural and forest aerosols, displaying 405 strong environmental adaptability (Nunez et al., 2021; Gusareva et al., 2019; Souza et al., 2021). Their widespread distribution suggests remarkable resilience to harsh environmental stressors, enabling them to survive and maintain culturability (Hu et al., 2018; Hu et al., 2020).

Most bacterial genera cultured from the atmosphere in various worldwide investigations are Gram-positive bacteria, with *Bacillus* as one of the most abundant genera (Yoo et al., 2019; Maki et al., 2010; Hua et al., 2007). The *Bacillus* genus has 410 often been identified as a dominant taxon in dust aerosols (Rossi et al., 2024; Péguilhan et al., 2023; Maki et al., 2022). For instance, *B. subtilis* has been found to increase significantly during dust events and maintain culturability. *B. subtilis* is known for its high adaptability to diverse extreme conditions, e.g., salt- and temperature-tolerant, allowing it to survive in a wide range of environments (Losick, 2020; Liu et al., 2018; Maki et al., 2022). The versatile metabolic capacity may play a physiological role in facilitating bacterial survival under harsh environments. Previous studies demonstrated that *Bacillus* sp. 415 3B6 was able to efficiently biotransform sugars into extracellular polymeric substances to protect this bacterium under hostile environment conditions, including cold temperature, radical exposure, and freeze processes (Matulová et al., 2014).

Additionally, members of the *Bacillus* genus are known for their ability to form endospores, enabling them to withstand atmospheric stressors and survive long-range transport (Nicholson et al., 2000).

Pantoea is a highly diverse genus found in both aquatic and terrestrial environments (Walterson et al., 2015; Murillo-Roos et al., 2022). *Pantoea* species exhibit a robust oxidative stress response by synthesizing enzymes such as catalase, superoxide dismutase, and peroxidase (Dahiya et al., 2024; Tambong, 2019). *Pseudomonas* species are well-adapted to atmospheric conditions, and they often oxidize common atmospheric volatile organic compounds, including formaldehyde and methanol (Husarova et al., 2011). *Streptomyces* is a dominant genus in aerosols from various megacities and background areas, especially in winter (Chen et al., 2021a; Petroselli et al., 2021; Li et al., 2019). Furthermore, *Streptomyces* has been widely reported in aerosols from European deserts and urban sewage treatment plants (Núñez et al., 2024; Zhan et al., 2024). *Streptomyces* is a drought-tolerant bacterial genus with spore-forming ability (Taketani et al., 2016). Their spores can enter a stable and quiescent state under environmental stress, enabling survival in diverse natural conditions (Naylor et al., 2017).

In this study, *Aspergillus* and *Penicillium* were the dominant fungal genera isolated. The spores of these species contain pigments, and most fungi produce secondary metabolites that include various pigments. Specifically, the exometabolites of *Penicillium* and *Talaromyces* were enriched with pigment compounds, particularly CRAM-like molecules with CHO elemental compositions (Fig. 6a and 6b). Some aliphatic/peptide-like and aromatic-like compounds were biotransformed into CRAM-like pigment molecules ($C_{14}H_{10}O_6$, $C_{23}H_{24}O_7$, $C_{26}H_{29}NO_8$, $C_{14}H_{10}O_6$, and $C_{15}H_{10}O_5$) (Fig. 7c and 7d). Microbial pigments are chemically diverse, including flavonoids, isoprenoids, porphyrins, N-heterocyclics, and polyketides (Contreras-Machuca et al., 2022; Morales-Oyervides et al., 2020; Venkatachalam et al., 2018). Recent studies have highlighted the potential of *Penicillium* and *Talaromyces* as robust producers of natural pigments, noted for their thermal, pH, and light stability (Ugwu et al., 2021; Akilandeswari and Pradeep, 2016). Many microorganisms isolated from atmospheric aerosols and rainwater were highly pigmented, potentially aiding their survival under intense ultraviolet exposure and photo-oxidative stress (Tong and Lighthart, 1997; Fahlgren et al., 2010; Fahlgren et al., 2015; Dassarma and Dassarma, 2018; Ziegelhoffer and Donohue, 2009). Additionally, many culturable halotolerant ice-nucleating bacterial and fungal strains isolated from coastal precipitation and aerosols could produce pigments (Beall et al., 2021). Microbial pigments can act as a protective layer for the cells, contributing to their long-distance atmospheric transport and adaptation to extreme environments.

4.2 Potential impacts of atmospheric microbial metabolites on atmospheric and marine organic matter

Molecules produced by culturable atmospheric microorganisms predominantly consisted of compounds with high O/C ratios and low H/C ratios, indicating a higher degree of oxidation (Fig.2 and 3). These findings align with previous studies, which suggest that organic compounds with lower O/C and higher H/C ratios are preferentially decomposed during microbial biotransformation, resulting in the production of oxygen-rich structures (Che et al., 2021; Gu et al., 2023). As microorganisms metabolism processes break down complex organic matter, oxygen-containing functional groups accumulate, signifying oxidative processes (Yuan et al., 2017).

Microorganisms consume molecules with high H/C ratios and labile molecules, leading to the generation of lower molecular weight organic molecules with lower H/C ratios (Ni et al., 2024). The decreased H/C ratios indicate a reduced presence of

aliphatic hydrocarbons, suggesting that these microorganisms may contribute to forming complex compounds with lower volatility (Li et al., 2021; Abbasian et al., 2015). These results underscore microorganisms replace H atoms with oxygen-rich functional groups such as carboxyl (COOH) and aldehyde (COH) groups during the oxidative degradation of DOM (Labrie et al., 2022). Therefore, the elevated O/C ratios indicate that atmospheric microorganisms may significantly enhance the oxidation of organic matter in clouds and aerosols, potentially influencing the organic carbon budget in atmospheric systems. The bacterial exometabolites predominantly consisted of lipid-like, aliphatic/peptide-like, CRAM-like, and tannin-like/highly oxygenated compounds (Fig. 2b). In contrast, the fungal exometabolites were mainly aliphatic/peptide-like and CRAM-like compounds (Fig. 3b). Notably, CRAM-like compounds were ubiquitous and the most abundant across both bacterial and fungal exometabolites, underscoring their pivotal role in microbial metabolic processes. CRAM-like compounds are an essential component of marine DOM and are produced and consumed by heterotrophic bacteria, which transform recalcitrant DOM (rDOM), as demonstrated in laboratory studies of the model diatom *Skeletonema dohrnii* (Liu et al., 2023c; Liu et al., 2021b). Additionally, long-term (220 days) macrocosm experiments at 16–22°C with mixed cultures of coastal seawater and diatom lysate have demonstrated that bacteria and archaea are key producers of rDOM, with CRAM-like compounds being a central component (He et al., 2022). Our findings align with these results, confirming that CRAM-like compounds are significant exometabolites of common atmospheric microorganisms. Consistent with these findings, incubation studies conducted on cloud water collected at the Puy de Dôme Observatory at 15°C in the dark revealed that microbial products are predominantly composed of lipid-like (16.5%), aliphatic/peptide-like (14.3%), and CRAM-like (44.7%) compounds, which closely aligns with the results of this study (Bianco et al., 2019). Moreover, after dark incubation for 30 days at room temperature, rainwater from coastal storms produced molecules primarily in the form of aliphatic/peptide-like and CRAM-like compounds (Mitra et al., 2013), similar to the products of microorganisms in this study. These results suggest that atmospheric microorganisms could contribute to the biotransformation of rDOM as it descends into marine environments.

4.3 Diverse metabolic processes and products of atmospheric microorganisms

Atmospheric microorganisms, including various bacterial and fungal strains, exhibit remarkable diversity in their metabolic processes and the products they generate. Analysis of exometabolites from different microbial species has revealed distinct molecular characteristics that underscore their unique metabolic pathways. The metabolomic profiles of airborne bacteria showed notable differences in metabolite production; however, CHON compounds accounted for over 50% of the total molecular formulas identified for all strains (Fig. 4). Similar findings were observed in the actual atmospheric environment, that the interactions between alive bacteria (*B. subtilis*, *Pseudomonas putida*, and *Enterobacter hormaechei*) and ·OH radicals in clouds contributed a significant amount of CHON compounds, comprising over 50% of the water-soluble compounds (Liu et al., 2023a).

A large number of CHONS compounds, belonging to aliphatic/peptide-like molecules, were detected in the metabolites of the Gram-negative bacteria *Stenotrophomonas* sp. and *P. baetica*, accounting for 40.6% (55.3% of intensity) and 33.2% (61.8% of intensity), respectively (Fig. 4b and S5). These compounds, including extracellular polymeric substances, play a crucial role in microbial survival by forming protective biofilms and contributing to the atmospheric biogeochemical cycle. The most

485 frequently detected genus in the atmosphere, *Pseudomonas*, is a key producer of biosurfactants, which can impact atmospheric chemistry (e.g., secondary organic formation) and modify cloud microphysics by enhancing cloud condensation nuclei activation owing to their exceptional ability to reduce surface tension (Delort et al., 2010).

Building on prior studies, some *Stenotrophomonas* spp. are known to synthesize a range of biologically active compounds, including a variety of antibiotic enzymes such as β -lactams, aminoglycosides, and macrolides, alongside chitinases, lipases, 490 and proteases (Ryan et al., 2009; Peleg and Abbott, 2015; Wang et al., 2018). These findings suggest that many of the CHONS compounds identified in the exometabolites are likely proteases or their degradation products, underscoring the pivotal role of *Stenotrophomonas* in protein turnover and nutrient cycles in the land/water-air interfaces (Liu et al., 2016). Furthermore, the molecular composition of the exometabolites pointed to an enrichment of amino acid metabolic pathways, like tyrosine metabolism (KO00350) and histidine metabolism (KO00340) (Fig. 8a). These pathways, coupled with the known reactivity of 495 amino acids, highlight the importance of considering the amino acid presence and transformation when investigating the chemical composition and oxidative processes occurring within cloud water (Bianco et al., 2016). This deeper understanding of bacterial exometabolites in atmospheric conditions provides new insights into the role of microorganisms in cloud chemistry and broader biogeochemical cycles.

Significant variations were observed in the metabolic products of different fungi: yeasts produced the highest diversity of 500 molecules, while molds produced more homogeneous compounds, with certain molecules having very high intensities (Fig. 6). *R. mucilaginosa*, as an essential biotechnological yeast, is a typical platform strain that can produce many functional bioproducts (Li et al., 2022). *R. mucilaginosa* was predominantly involved in carbohydrate metabolism (Fig. 8b). In this study, several saturated fatty acids, including myristic acid ($C_{14}H_{28}O_2$), stearic acid ($C_{18}H_{36}O_2$), and palmitic acid ($C_{16}H_{32}O_2$), and unsaturated fatty acids including linoleic acid ($C_{18}H_{32}O_2$) and linolenic acid ($C_{18}H_{30}O_2$), were detected. These organic acids 505 were also abundantly present in atmospheric aerosols and may affect the atmospheric chemical and physical processes, e.g., cloud formation (Mkoma and Kawamura, 2013; Balducci and Cecinato, 2010; Raymond and Pandis, 2002). Notably, palmitic acid, stearic acid, and linoleic acid are potential molecular tracers for estimating the contribution of cooking emissions to organic aerosols (Cheng and Yu, 2020; Ma et al., 2023). However, our findings reveal that fatty acids were also abundant in the metabolites of *Rhodotorula*, suggesting that microorganisms may contribute to atmospheric fatty acid levels. This 510 highlights the need to consider microbial sources when assessing the composition of atmospheric organic aerosols.

Among airborne fungi, the genera *Aspergillus*, *Penicillium*, and *Talaromyces*, which are classified as mold fungi, are acknowledged as some of the most chemically prolific organisms. These genera can synthesize a wide variety of secondary metabolites (exometabolites) (Frisvad, 2015; Zhai et al., 2016; Morales-Oyervides et al., 2020). They produce specialized compounds such as polyketides, non-ribosomal peptides, and terpenes, further showcasing their metabolic versatility and 515 biochemical complexity (Frisvad, 2015; Adelusi et al., 2022). This remarkable chemodiversity highlights their ecological significance in atmospheric biogeochemistry. *A. niger* is ubiquitous in the environment and plays a significant role in the global carbon cycle (Baker, 2006; Schuster et al., 2002; Šimonovičová et al., 2021). It is rich in genetic and metabolic diversity, acting as one of the most important microorganisms used in biotechnology (Sun et al., 2007; Jørgensen et al., 2011). In its

natural growth state, *A. niger* possesses large cryptic biosynthetic gene clusters (BGCs), which synthesize a wide range of extracellular enzymes to degrade special biopolymers in the environment, thus allowing the fungus to obtain nutrients (Yu et al., 2021; Romsdahl and Wang, 2019). Anthraquinones like C₁₅H₁₂O₅, C₁₅H₁₀O₅, and C₁₅H₁₀O₆ were detected in the product of *Penicillium* and *Talaromyces*, and these compounds can protect symbiotic plants from pests or pathogens (Etalo et al., 2018). This metabolic plasticity underscores their ecological importance and the potential for biotechnological applications in various industries. Given the ecological and biotechnological significance of secondary metabolites from fungi, further exploration of these exometabolites remains a priority in atmospheric microbial research.

5 Conclusions

This study unveiled the molecular characteristics of exometabolites produced by typical culturable microorganisms in the urban atmosphere, integrated with metabolic pathways, enriching the foundational data for untargeted studies of microbial exometabolites using FT-ICR MS. Vital metabolic processes for various bacteria and fungi were identified through mass-based transformation analysis and KEGG enrichment analyses. The culturable atmospheric bacteria were primarily *Pseudomonas* and *Bacillus*, and the predominant fungal genera were *Aspergillus* and *Penicillium*. Bacterial and fungal strains produce exometabolites with lower H/C ratios and higher O/C ratios, suggesting that airborne microorganisms have the potential to influence atmospheric oxidative capacity and the organic carbon budget. The typical bacteria largely contributed to CHON and CHONS compounds in their metabolism, whereas fungi mainly produced CHO compounds as exometabolites. The microbial exometabolites predominantly consisted of aliphatic/peptide-like and CRAM-like compounds. The unique molecules of each typical bacterial strain were highly oxidizable and can serve as indicator molecules for various bacteria. Microbial metabolic pathways varied significantly, with lipid metabolism and amino acid metabolism showing considerable variations among different bacteria, while carbohydrate metabolism and secondary metabolism differed markedly among fungi. *Talaromyces* sp. exhibited a unique metabolic process with exometabolites dominated by CHO compounds, primarily undergoing carboxylation and glyoxylate transformations. Additionally, aflatoxin biosynthesis was identified as the central metabolic pathway of *Talaromyces* sp., along with the biosynthesis of other secondary metabolites. These findings enhance our understanding of the potential roles of atmospheric microorganisms in atmospheric chemistry and biogeochemical cycles, especially providing support for potential metabolic processes that could occur if atmospheric microorganisms are deposited onto the oceanic or inland water surfaces. Future research should focus more on the deposition and activity of atmospheric microorganisms in natural environments and develop a comprehensive database of source profiles for typical atmospheric microbial exometabolites at the molecular level.

Data availability. The dataset for this paper is available upon request from the corresponding authors (huwei@tju.edu.cn; fupingqing@tju.edu.cn).

Author contribution. WH, PF, and RJ designed the study and the experiments. RJ, MN, and PD executed experiments. RJ, MS, DL, and ZH analyzed the data. RJ and WH visualized the data, and wrote the draft manuscript with input from all of the authors. All of the authors discuss, edit, and approved the manuscript.

Competing interests. The authors declare that they have no conflict of interests.

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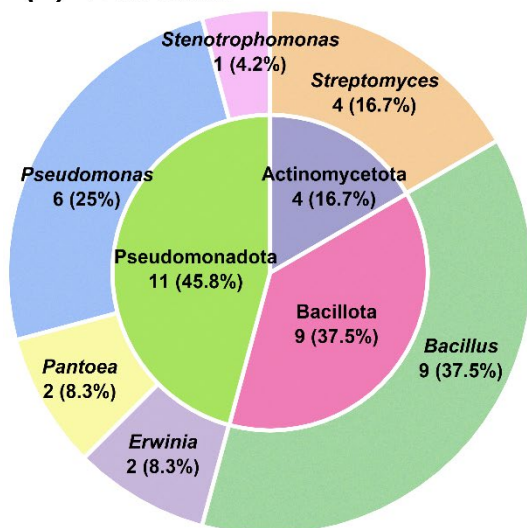
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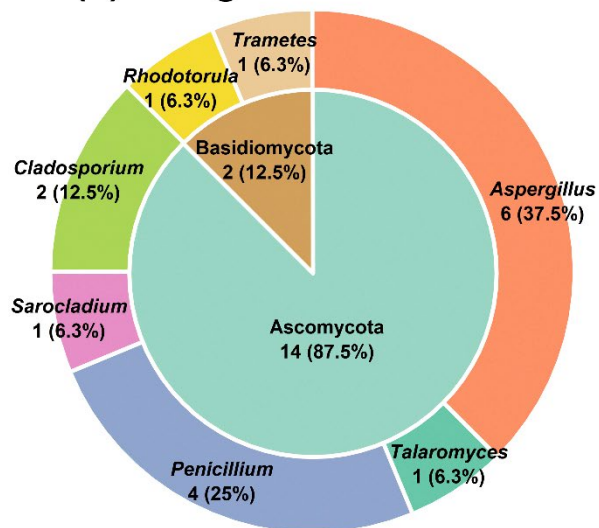
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(a) Bacteria



(b) Fungi



970 **Figure 1.** The distribution of culturable bacterial (a) and fungal (b) taxa at the phylum (inner pie charts) and genus (external pie charts) levels.

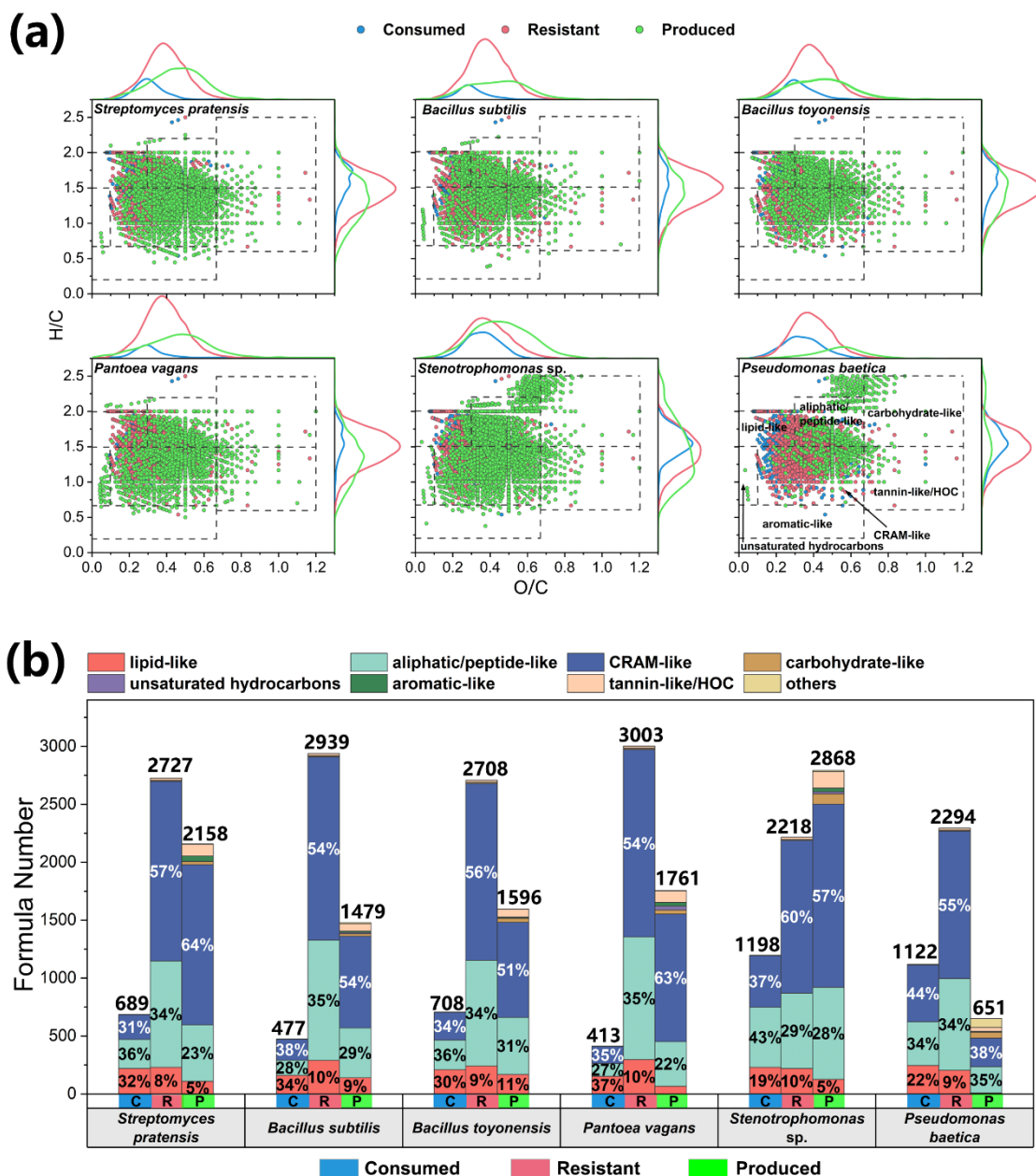


Figure 2. Changes in molecular compositions of culture media during the metabolic processes of six typical atmospheric culturable bacteria. (a) Van Krevelen diagrams illustrate changes in different categories of organics in the media after incubation. The top three Van Krevelen diagrams represent the chemical composition of Gram-positive bacteria, the bottom three Van Krevelen diagrams correspond to Gram-negative bacteria. (b) The stacked bar charts indicate the formula numbers of different categories of consumed (labeled as C), resistant (R), and produced (P) organic matter.

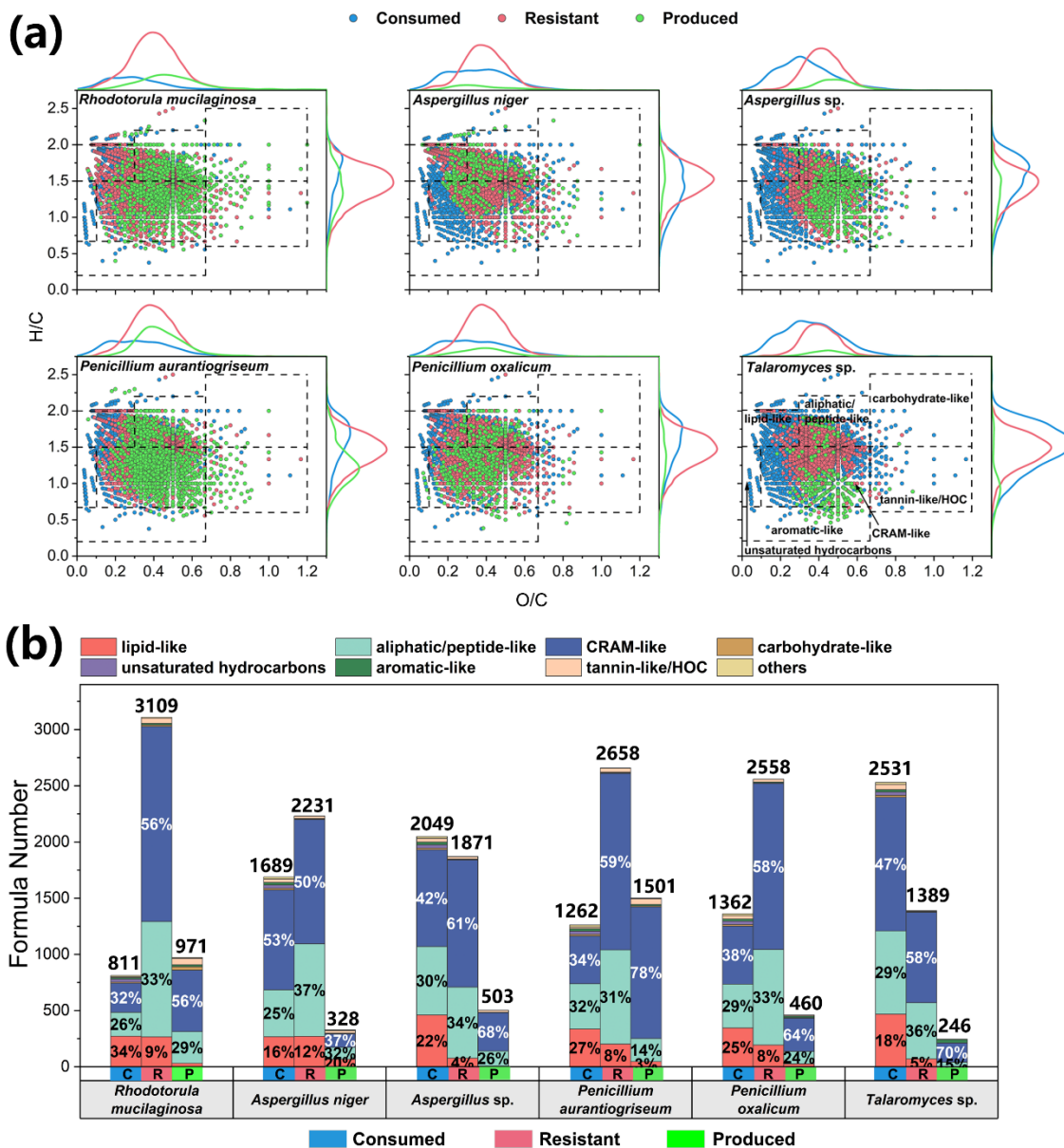


Figure 3. Changes in molecular compositions of culture media during the metabolic processes of six typical atmospheric culturable fungi. (a) Van Krevelen diagrams illustrate changes in different categories of organics in the media after incubation. (b) The stacked bar charts indicate the formula numbers of different categories of consumed (labeled as C), resistant (R), and produced (P) organic matter.

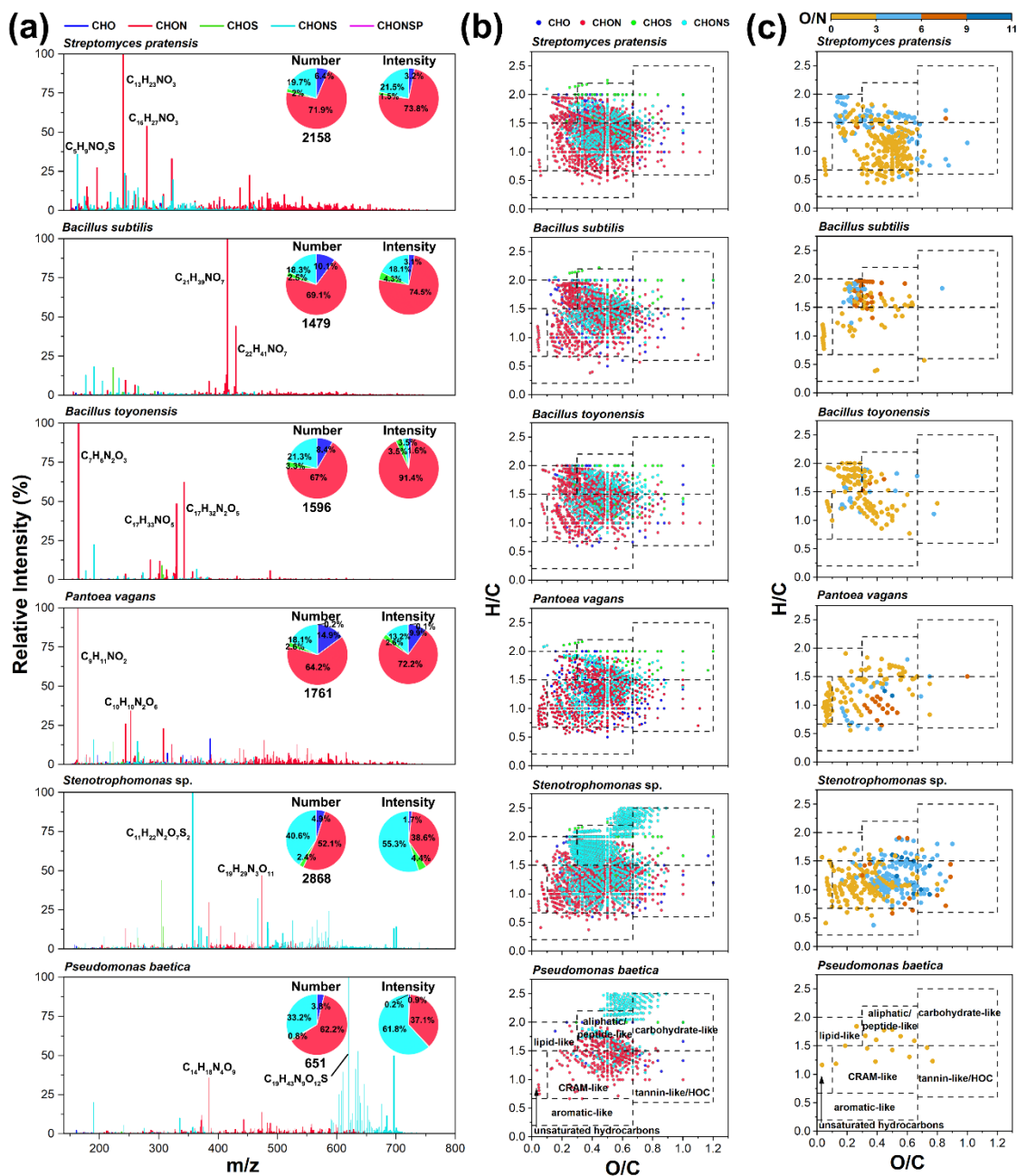


Figure 4. Molecular characteristics of bacterial exometabolites. (a) Mass spectra and pie charts show the distribution of m/z values and elemental compositions in bacterial exometabolites. (b) Van Krevelen diagrams show the molecular distribution of bacterial exometabolites classified by elemental composition (CHO, CHON, CHOS, and CHONS). (c) Van Krevelen diagrams reveal the distribution of unique CHON compounds with different O/N ratios for each bacterial strain.

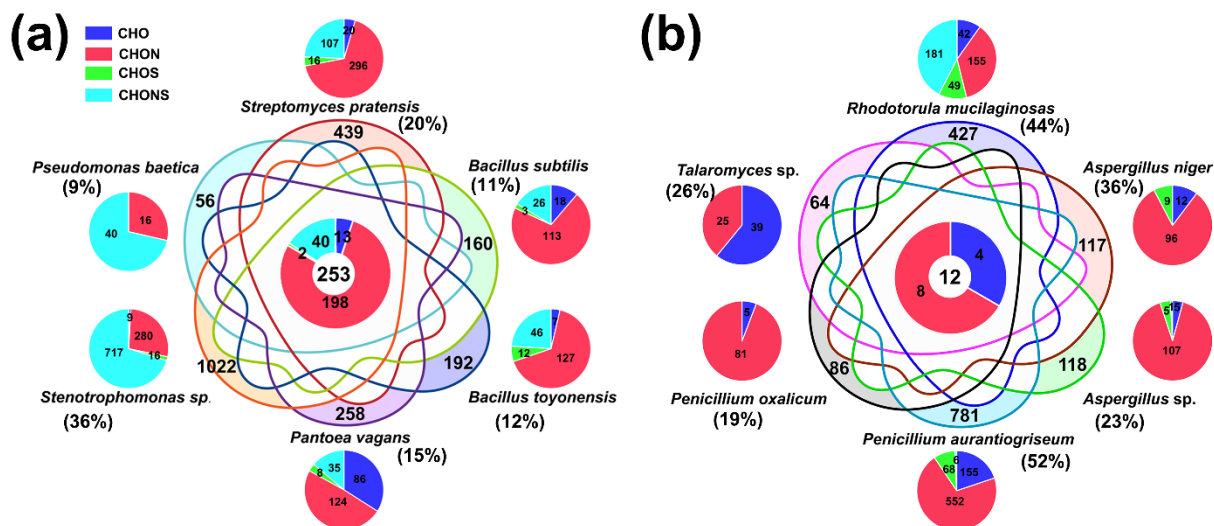


Figure 5. Differences in exometabolites among different typical bacteria (a) and fungi (b). The Venn diagrams display the numbers and percentages of shared and unique formulas for six typical bacterial or fungal strains. The percentages represent the number fraction of unique formulas relative to the total formulas for each strain. The donut diagram in the center and pie charts show the elemental compositions of shared and unique formulas for each strain.

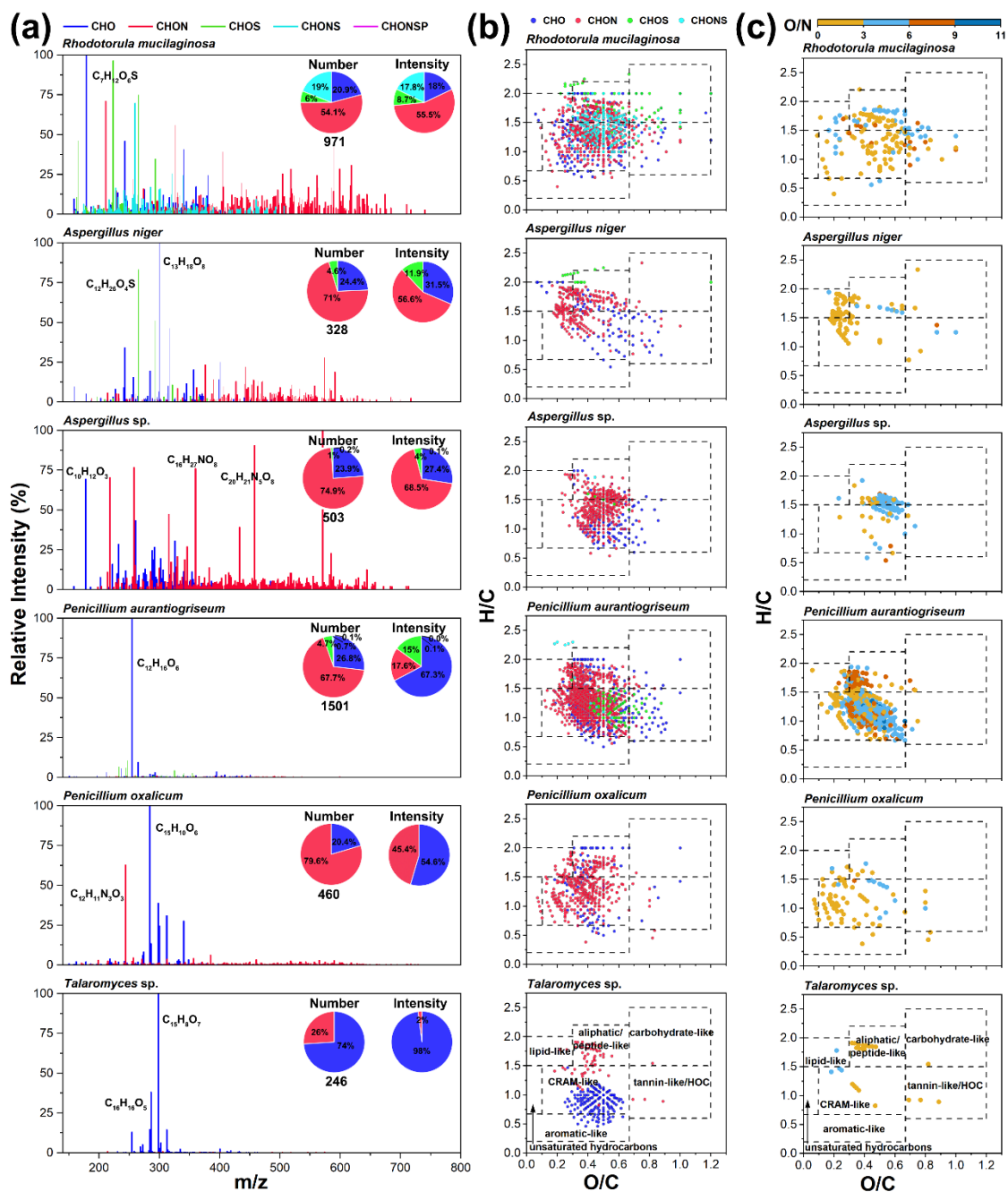


Figure 6. Molecular characteristics of fungal exometabolites. (a) Mass spectra and pie charts show the distribution of m/z values and elemental compositions of fungal exometabolites. (b) Van Krevelen diagrams show the molecular distribution of fungal exometabolites classified by elemental composition (CHO, CHON, CHOS, and CHONS). (c) Van Krevelen diagrams reveal the distribution of unique CHON compounds with different O/N ratios for each fungal strain.

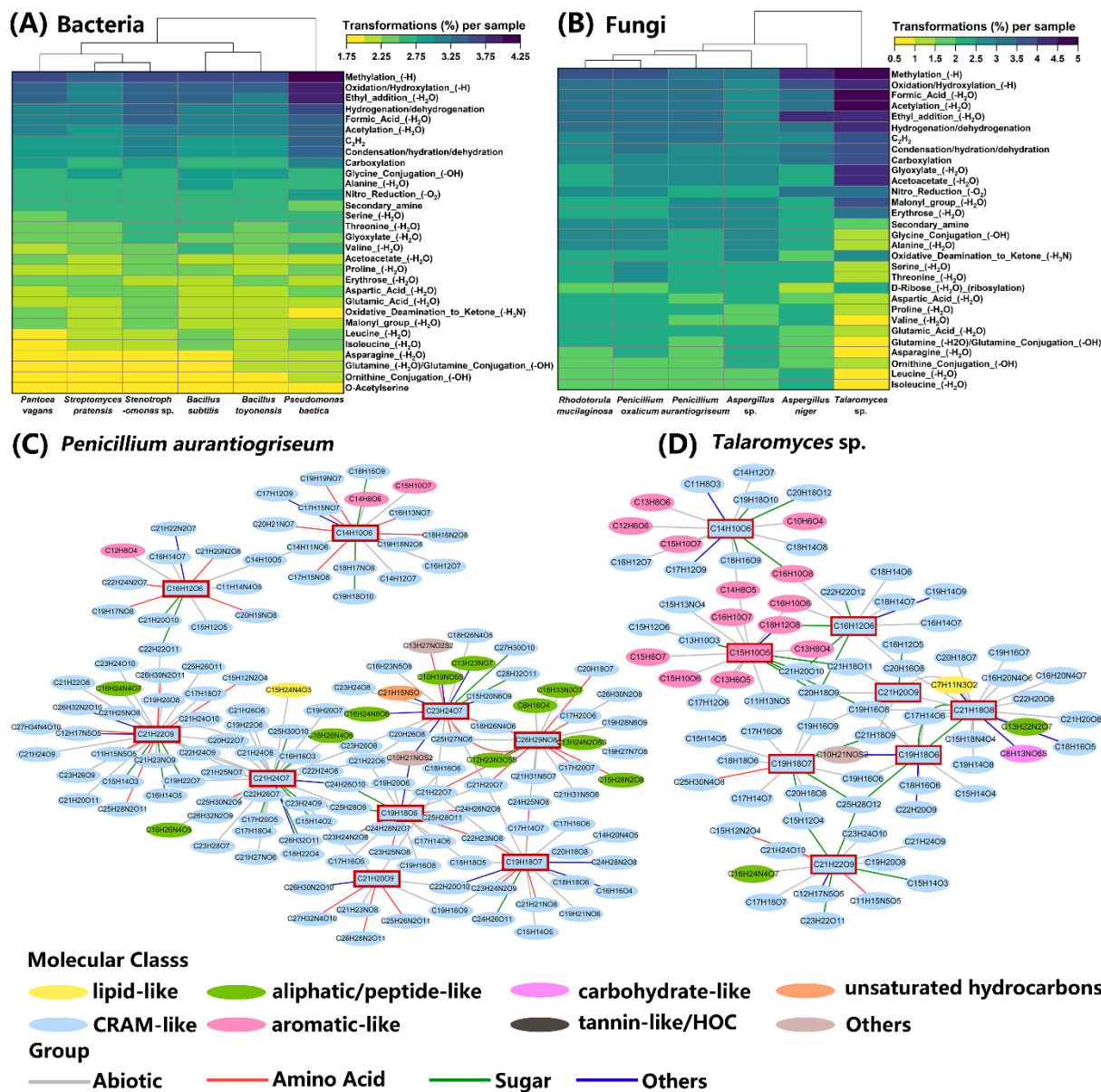
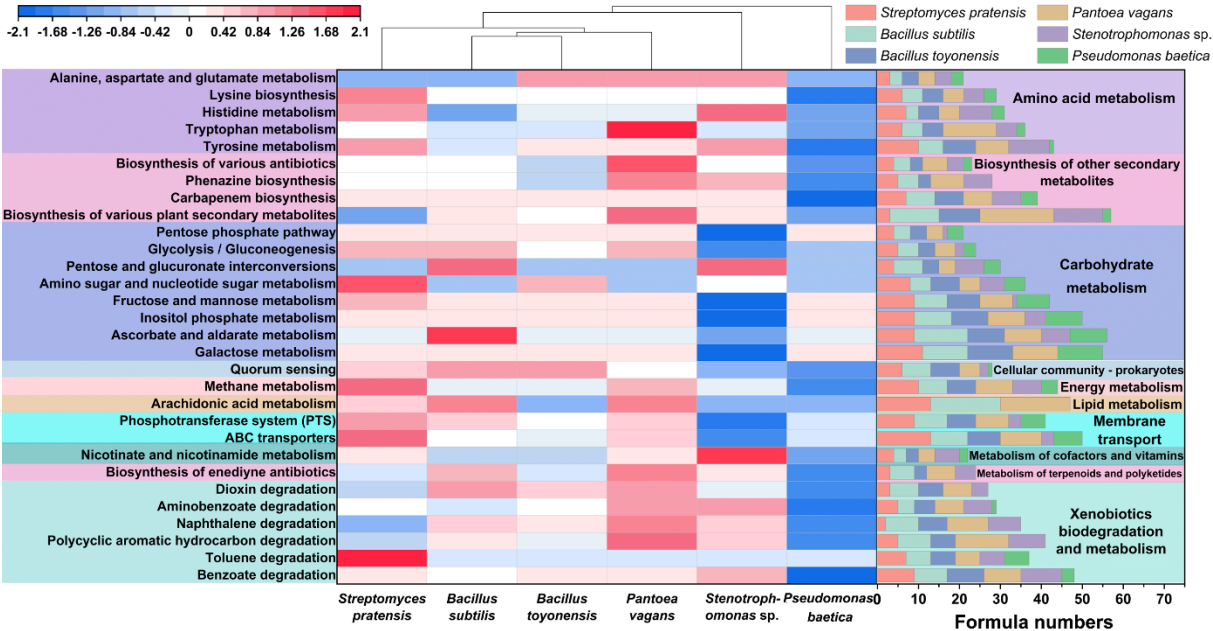


Figure 7. The types and networks of biochemical transformations for bacteria and fungi. Heatmaps show the top 30 transformation types in terms of relative abundance for bacteria (a) and fungi (b). Molecular networks illustrate the transformations for the major pigment formulas (Table S8) of *P. aurantiogriseum* (c) and *Talaromyces* sp. (d).

(a) Bacteria



(b) Fungi

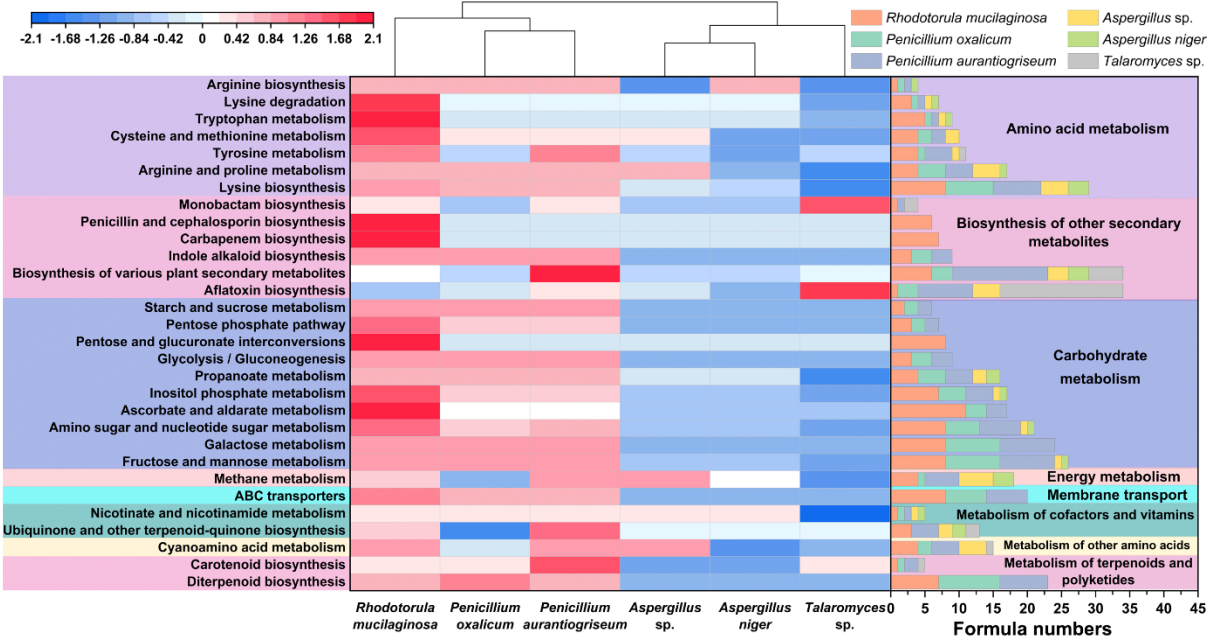


Figure 8. The KEGG metabolic pathways for typical airborne bacterial (a) and fungal (b) strains. Heatmaps illustrate the normalized relative abundance (top 30) of KEGG tertiary pathways of bacterial and fungal strains. Stacked bar diagrams show the formula numbers annotated to microbial exometabolites in each pathway.

Supplement of

Exometabolomic exploration of culturable airborne microorganisms from an urban atmosphere

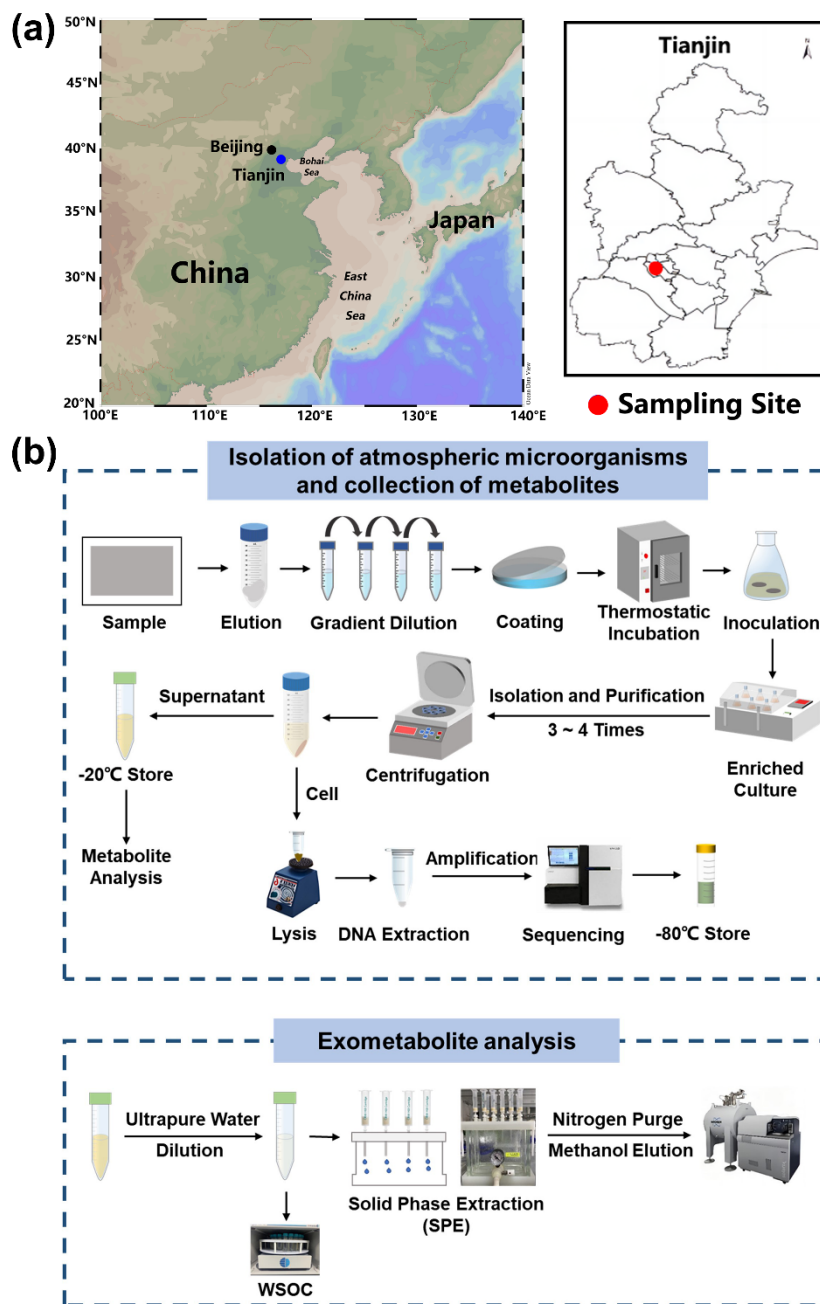
Rui Jin¹, Wei Hu^{1,2*}, Peimin Duan¹, Ming Sheng¹, Dandan Liu¹, Ziyi Huang¹, Mutong Niu¹, Libin Wu¹,
5 Junjun Deng¹, Pingqing Fu^{1,3*}

¹Institute of Surface-Earth System Science, School of Earth System Science, Tianjin University, Tianjin 300072, China

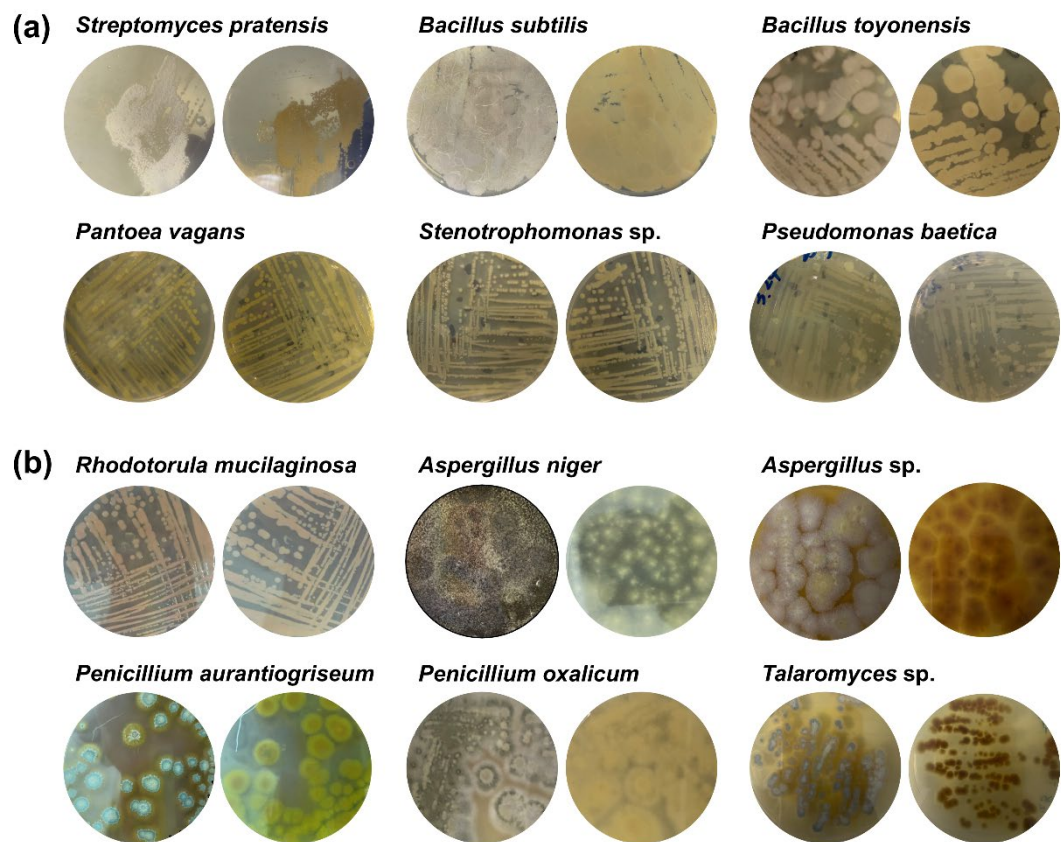
²Tianjin Bohai Rim Coastal Earth Critical Zone National Observation and Research Station, Tianjin University, Tianjin 300072, China

³Tianjin Key Laboratory of Earth Critical Zone Science and Sustainable Development in Bohai Rim, Tianjin University,
10 Tianjin 300072, China

Correspondence to: Wei Hu (huwei@tju.edu.cn); Pingqing Fu (fupingqing@tju.edu.cn).



15 **Figure S1.** The location of culturable airborne microorganisms and experimental procedures. (a) The map shows the location of Tianjin city and the urban sampling site in the Nankai district, Tianjin (the map is from Ocean Data View). (b) Experimental procedures include the isolation of atmospheric microorganisms, collection of metabolites, and exometabolite analysis.



20 **Figure S2.** The representative bacterial (a) and fungal (b) strains isolated atmospheric microorganisms.

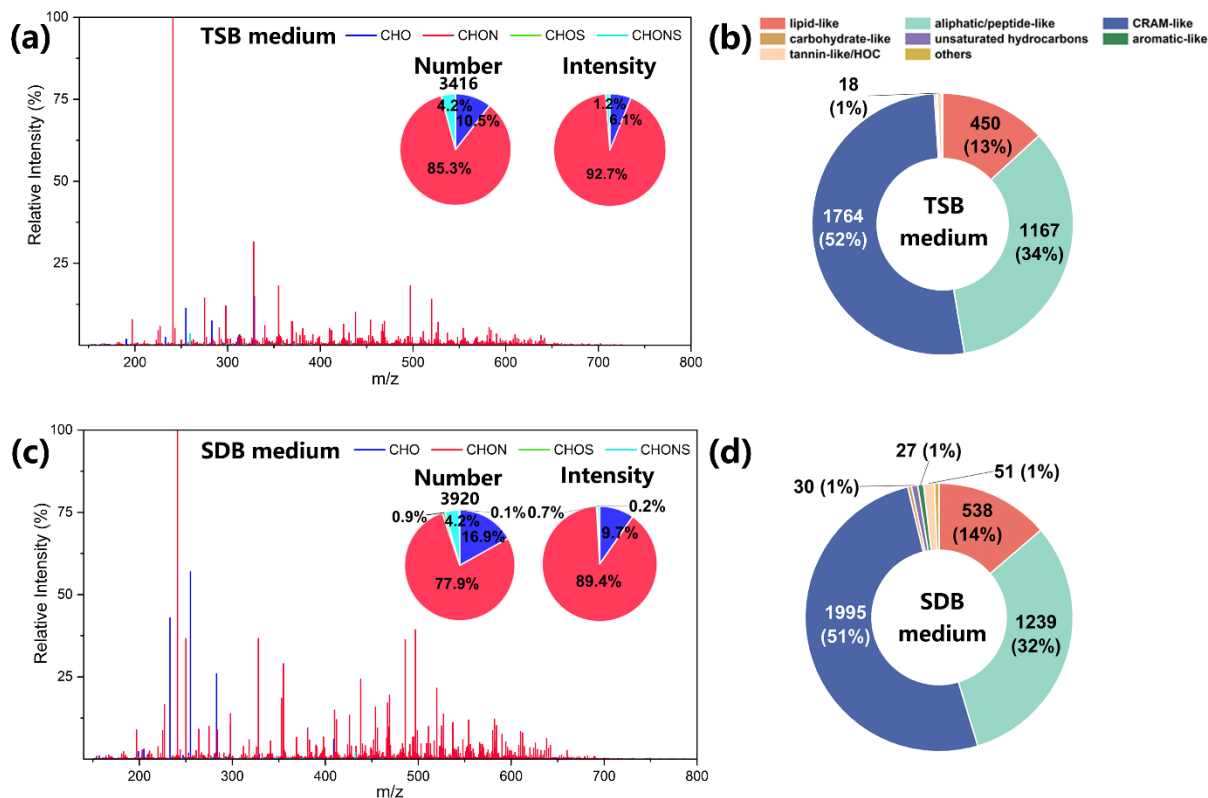


Figure S3. Mass spectra and different categories of organic compounds in the initial culture broths. (a-b) The mass spectrum and elemental composition of TSB used for bacterial growth (a), and the formula numbers of different categories of organic matter for TSB (b). (c-d) The mass spectrum and elemental composition of SDB used for fungal growth (c), and the formula numbers of different categories of organic matter for SDB (d).

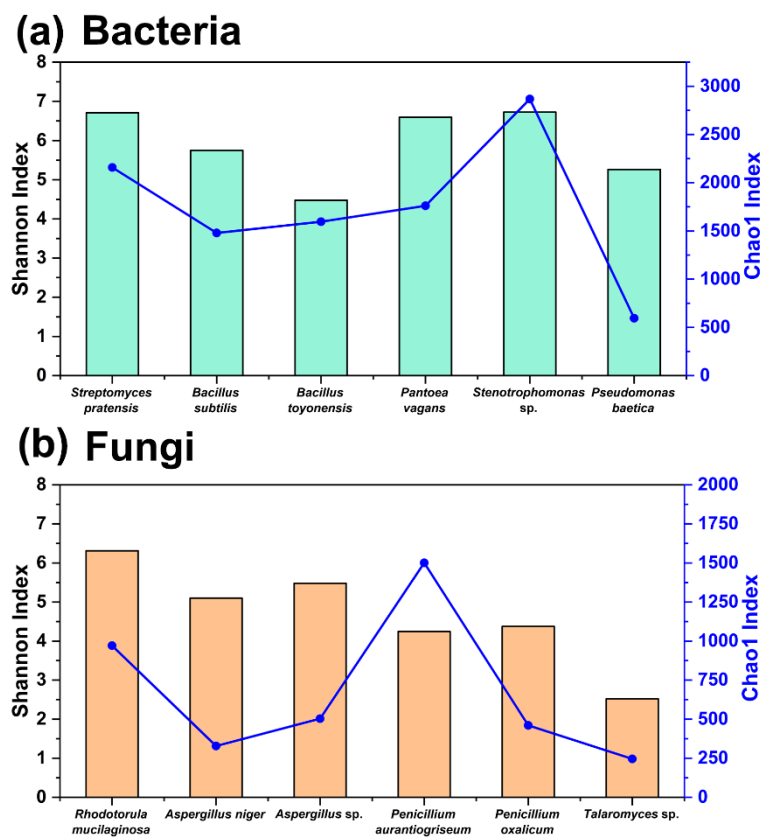


Figure S4. The molecular diversity of bacterial (a) and fungal (b) exometabolites.

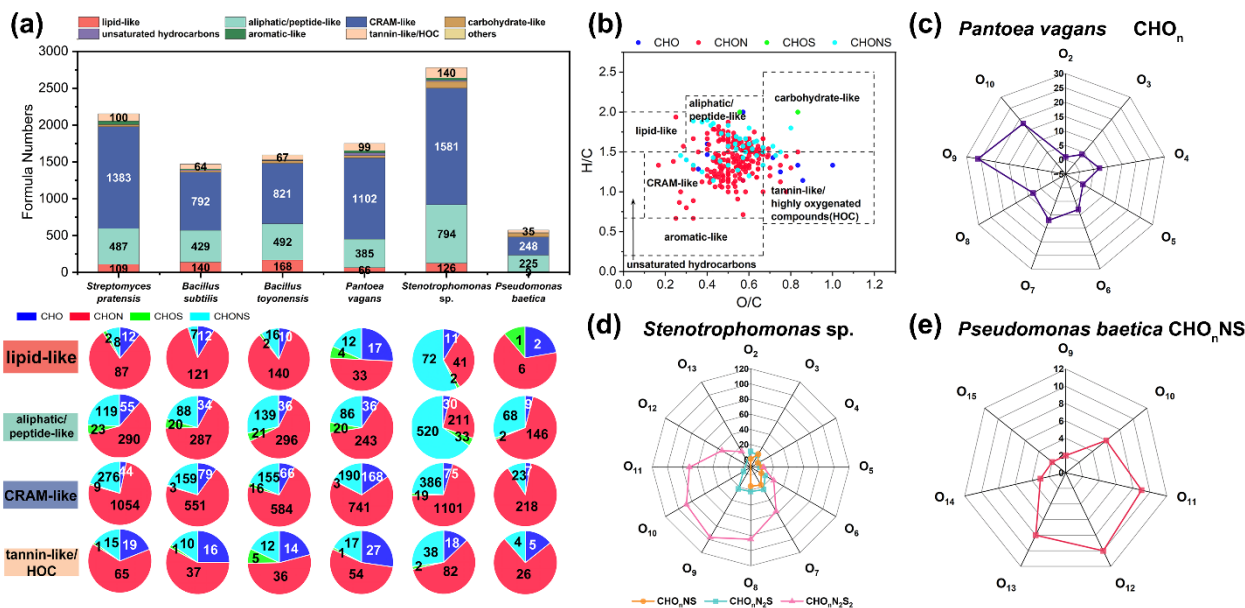


Figure S5. The formula categories of bacterial exometabolites and differences in the molecular composition of shared and unique exometabolites among bacterial strains. (a) Stacked bars illustrate formula categories based on H/C and O/C ratios in bacterial exometabolites, and pie charts illustrate the elemental compositions of high abundance categories. (b) van Krevelen diagrams illustrate the shared molecules. Radar maps show the distribution of oxygen number of unique CHO compounds in exometabolites from *Pantoea vagans* (c) and the distribution of oxygen number of unique CHONS compounds in exometabolites from *Stenotrophomonas sp.* (d) and *Pseudomonas baetica* (e).

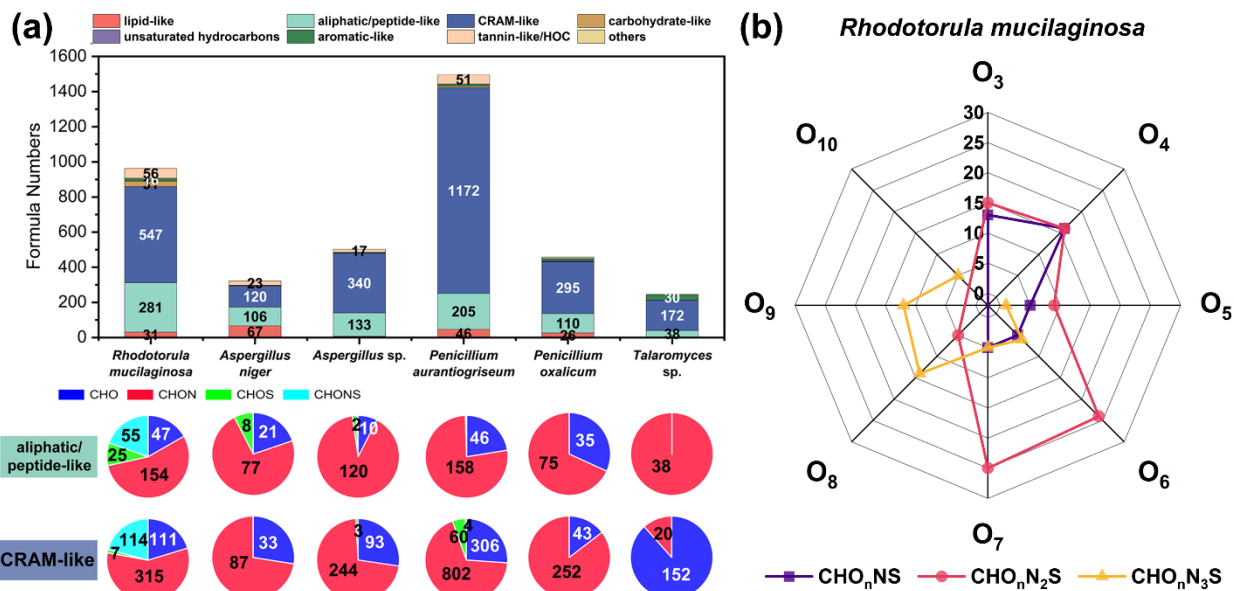


Figure S6. The formula categories of fungal exometabolites (a) and the distribution of oxygen number for unique CHONS compounds in exometabolites from *Rhodotorula mucilaginosa* (b). Stacked bars illustrate formula categories based on H/C and O/C ratios in fungal exometabolites, and pie charts illustrate the elemental compositions of high abundance categories.

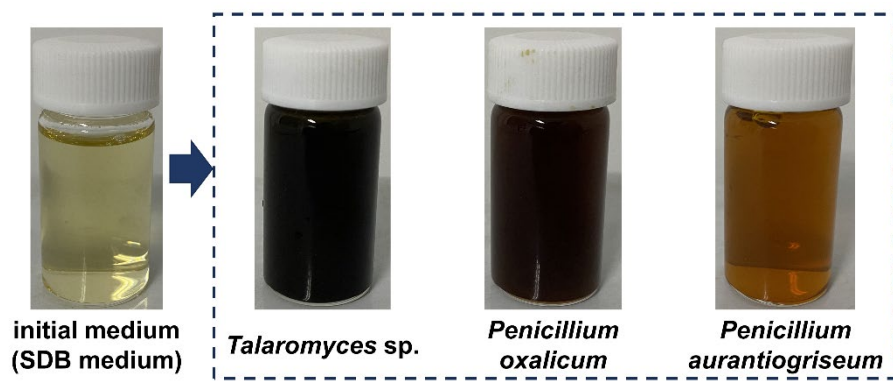


Figure S7. Changes in the supernatant of the culture media for the genera *Penicillium* and *Talaromyces* after 14 days.

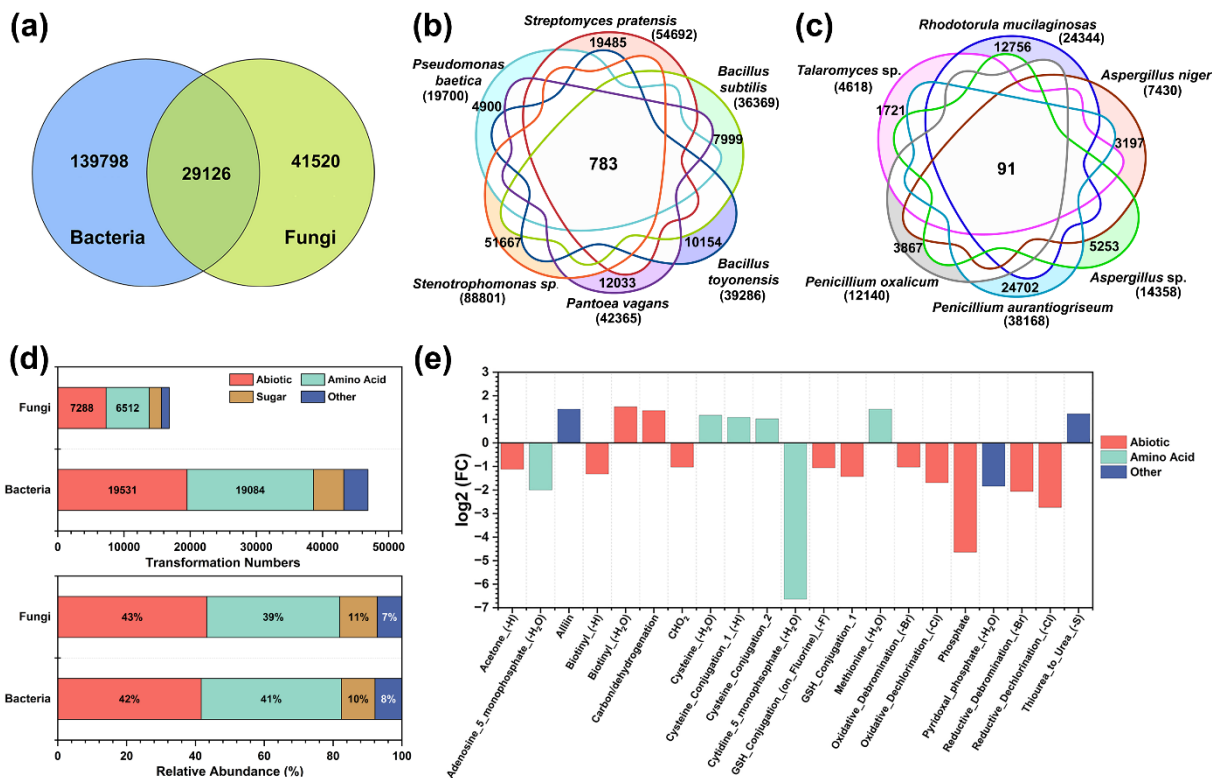


Figure S8. The differences in molecular transformations for bacterial and fungal species. (a-c) Venn diagrams display the numbers of shared and unique potential molecular transformations of bacteria and fungi (a), typical bacteria (b), and typical fungi (c). Each strain's total number of transformations is labeled below the species name. (d) Bar diagrams show the grouped distribution of the transformation numbers and the relative abundance of bacteria and fungi. (e) Bar diagram illustrates the distribution of transformation types with significant differences (Fold Change (FC) ≥ 2 or ≤ 0.5) between bacteria and fungi. $\text{Log}_2(\text{FC}) \geq 0$ indicates that this transformation type is more significantly represented in bacteria.

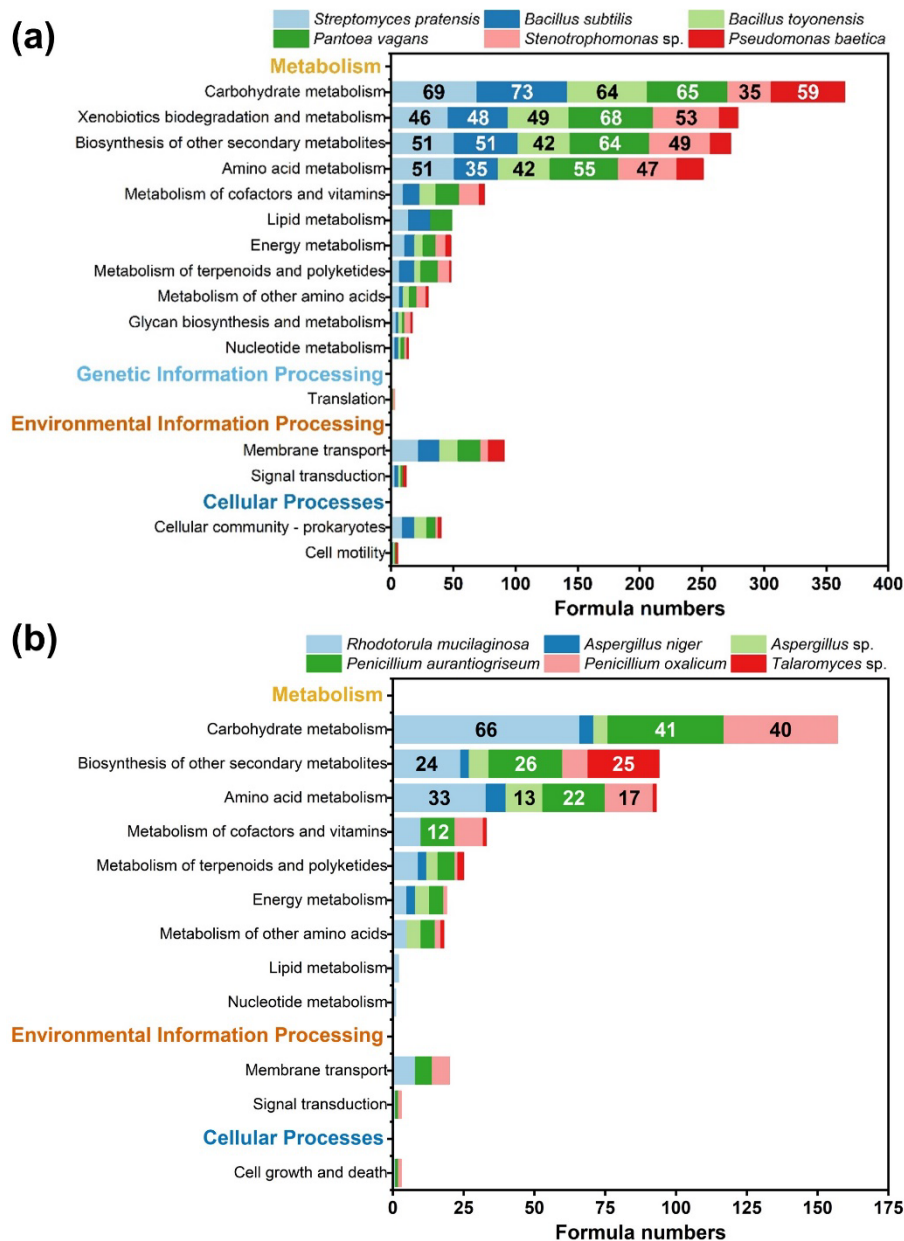


Figure S9. The KEGG metabolic pathways of typical bacterial (a) and fungal (b) strains enriched and analyzed based on their exometabolites, including both primary and secondary pathways.

55 **Table S1** Compositions of the media used for isolation of culturable bacteria and fungi in the urban atmosphere.

Component	Tryptic soy agar (TSA)	Sabouraud dextrose agar (SDA)
Tryptone	15 g	-
Soy peptone	5 g	-
NaCl	5 g	-
Peptone	-	10 g
Glucose	-	40 g
Agar	15 g	20 g
Double distilled water	1000 mL	1000 mL

Table S2 Identities of cultivable bacteria derived from aerosol samples.

Isolate ID	Isolated strains	BLAST Identity	% Identity	Phylum	Class	Order	Family
B1	<i>Bacillus</i> sp. TJB1	<i>Bacillus</i> sp.	99.93%	Bacillota	Bacilli	Bacillales	Bacillaceae
B2	<i>Pantoea vagans</i> TJB2	<i>Pantoea vagans</i>	100.00%	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Erwiniaceae
B3	<i>Streptomyces thermoviolaceus</i> TJB3	<i>Streptomyces thermoviolaceus</i>	100.00%	Actinomycetota	Actinobacteria	Streptomycetales	Streptomycetaceae
B4	<i>Bacillus subtilis</i> TJB4	<i>Bacillus subtilis</i>	99.17%	Bacillota	Bacilli	Bacillales	Bacillaceae
B5	<i>Streptomyces thermoviolaceus</i> TJB5	<i>Streptomyces thermoviolaceus</i>	99.93%	Actinomycetota	Actinobacteria	Streptomycetales	Streptomycetaceae
B6	<i>Bacillus</i> sp. TJB6	<i>Bacillus</i> sp. Y1(2012)	99.93%	Bacillota	Bacilli	Bacillales	Bacillaceae
B7	<i>Bacillus subtilis</i> TJB7	<i>Bacillus subtilis</i>	100.00%	Bacillota	Bacilli	Bacillales	Bacillaceae
B8	<i>Erwinia</i> sp. TJB8	<i>Erwinia</i> sp.	100.00%	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Erwiniaceae
B9	<i>Erwinia</i> sp. TJB9	<i>Erwinia</i> sp.	100.00%	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Erwiniaceae
B10	<i>Streptomyces pratensis</i> TJB10	<i>Streptomyces pratensis</i>	99.12%	Actinomycetota	Actinobacteria	Streptomycetales	Streptomycetaceae
B11	<i>Streptomyces</i> sp. TJB11	<i>Streptomyces</i> sp. SYP-A7193	99.93%	Actinomycetota	Actinobacteria	Streptomycetales	Streptomycetaceae
B12	<i>Bacillus</i> sp. TJB12	<i>Bacillus</i> sp. 210_62	99.72%	Bacillota	Bacilli	Bacillales	Bacillaceae
B13	<i>Pseudomonas</i> sp. TJB13	<i>Pseudomonas</i> sp.	99.93%	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B14	<i>Pantoea vagans</i> TJB14	<i>Pantoea vagans</i>	99.32%	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Erwiniaceae
B15	<i>Pseudomonas</i> sp. TJB15	<i>Pseudomonas</i> sp. B14-6	100.00%	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B16	<i>Stenotrophomonas</i> sp. TJB16	<i>Stenotrophomonas</i> sp.	99.79%	Pseudomonadota	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae
B17	<i>Pseudomonas fluorescens</i> TJB17	<i>Pseudomonas fluorescens</i>	100.00%	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B18	<i>Bacillus toyonensis</i> TJB18	<i>Bacillus toyonensis</i>	100.00%	Bacillota	Bacilli	Bacillales	Bacillaceae
B19	<i>Pseudomonas</i> sp. TJB19	<i>Pseudomonas</i> sp.	99.71%	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B20	<i>Bacillus halotolerans</i> TJB20	<i>Bacillus halotolerans</i>	100.00%	Bacillota	Bacilli	Bacillales	Bacillaceae
B21	<i>Bacillus proteolyticus</i> TJB21	<i>Bacillus proteolyticus</i>	100.00%	Bacillota	Bacilli	Bacillales	Bacillaceae
B22	<i>Bacillus safensis</i> TJB22	<i>Bacillus safensis</i>	100.00%	Bacillota	Bacilli	Bacillales	Bacillaceae
B23	<i>Pseudomonas baetica</i> TJB23	<i>Pseudomonas baetica</i>	100.00%	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae
B24	<i>Pseudomonas</i> sp. TJB24	<i>Pseudomonas</i> sp.	99.85%	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae

Table S3 Identities of cultivable fungi derived from aerosol samples.

Isolate ID	Isolated strains	BLAST Identity	% Identity	Phylum	Class	Order	Family
F1	<i>Trametes elegans</i> TJF1	<i>Trametes elegans</i>	100.00%	Basidiomycota	Agaricomycetes	Polyporales	Polyporaceae
F2	<i>Talaromyces</i> sp. TJF2	<i>Talaromyces</i> sp.	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae
F3	<i>Aspergillus niger</i> TJF3	<i>Aspergillus niger</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F4	<i>Aspergillus</i> sp. TJF4	<i>Aspergillus</i> sp.	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F5	<i>Aspergillus</i> sp. TJF5	<i>Aspergillus</i> sp.	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F6	<i>Aspergillus nidulans</i> TJF6	<i>Aspergillus nidulans</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F7	<i>Penicillium oxalicum</i> TJF7	<i>Penicillium oxalicum</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F8	<i>Sarocladium terricola</i> TJF8	<i>Sarocladium terricola</i>	100.00%	Ascomycota	Sordariomycetes	Hypocreales	Sarocladiaceae
F9	<i>Penicillium sumatraense</i> TJF9	<i>Penicillium sumatraense</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F10	<i>Penicillium aurantiogriseum</i> TJF10	<i>Penicillium aurantiogriseum</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F11	<i>Aspergillus</i> sp. TJF11	<i>Aspergillus</i> sp.	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F12	<i>Aspergillus carneus</i> TJF12	<i>Aspergillus carneus</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F13	<i>Cladosporium parahalotolerans</i> TJF13	<i>Cladosporium parahalotolerans</i>	100.00%	Ascomycota	Dothideomycetes	Cladosporiales	Cladosporiaceae
F14	<i>Rhodotorula mucilaginosa</i> TJF14	<i>Rhodotorula mucilaginosa</i>	100.00%	Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae
F15	<i>Penicillium cinnamopurpureum</i> TJF15	<i>Penicillium cinnamopurpureum</i>	100.00%	Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae
F16	<i>Cladosporium</i> sp. TJF16	<i>Cladosporium</i> sp.	99.42%	Ascomycota	Dothideomycetes	Cladosporiales	Cladosporiaceae

Table S4 The bacterial and fungal isolates used for exometabolomic studies.

Kingdom	Isolate ID	Isolated strains	Species	Category
Bacteria	B10	<i>Streptomyces pratensis</i> TJB10	<i>Streptomyces pratensis</i>	Gram-positive bacteria
	B4	<i>Bacillus subtilis</i> TJB4	<i>Bacillus subtilis</i>	Gram-positive bacteria
	B18	<i>Bacillus toyonensis</i> TJB18	<i>Bacillus toyonensis</i>	Gram-positive bacteria
	B2	<i>Pantoea vagans</i> TJB2	<i>Pantoea vagans</i>	Gram-negative bacteria
	B16	<i>Stenotrophomonas</i> sp. TJB16	<i>Stenotrophomonas</i> sp.	Gram-negative bacteria
	B23	<i>Pseudomonas baetica</i> TJB23	<i>Pseudomonas baetica</i>	Gram-negative bacteria
Fungi	F14	<i>Rhodotorula mucilaginosa</i> TJF14	<i>Rhodotorula mucilaginosa</i>	yeast
	F3	<i>Aspergillus niger</i> TJF3	<i>Aspergillus niger</i>	mold
	F5	<i>Aspergillus</i> sp. TJF5	<i>Aspergillus</i> sp.	mold
	F10	<i>Penicillium aurantiogriseum</i> TJF10	<i>Penicillium aurantiogriseum</i>	mold
	F7	<i>Penicillium oxalicum</i> TJF7	<i>Penicillium oxalicum</i>	mold
	F2	<i>Talaromyces</i> sp. TJF2	<i>Talaromyces</i> sp.	mold

Note: The species names instead of the strain names are used in the figures and tables to facilitate the description of the different isolates.

Table S5 Changes in the number of formulas after incubation for bacteria and fungi.

Media / Species	Culture nutrients	Consumed			Resistant			Produced			Total
		Number	H/C	O/C	Number	H/C	O/C	Number	H/C	O/C	Number
Before incubation											
TSB	-	-			-			-			3416
SDB	-	-			-			-			3920
After incubation											
<i>Streptomyces pratensis</i>	TSB	689	1.60	0.31	2727	1.46	0.39	2158	1.32	0.46	4885
<i>Bacillus subtilis</i>	TSB	477	1.56	0.30	2939	1.47	0.39	1479	1.43	0.44	4418
<i>Bacillus toyonensis</i>	TSB	708	1.58	0.32	2708	1.46	0.39	1596	1.44	0.44	4304
<i>Pantoea vagans</i>	TSB	413	1.58	0.30	3003	1.47	0.39	1761	1.32	0.45	4764
<i>Stenotrophomonas</i> sp.	TSB	1198	1.57	0.36	2218	1.44	0.39	2868	1.42	0.46	5086
<i>Pseudomonas baetica</i>	TSB	1122	1.53	0.34	2294	1.47	0.39	651	1.65	0.53	2945
Mean ± SE		768±133	1.57±0.01	0.32±0.01	2648±133	1.46±0.00	0.39±0.00	1752±301	1.43±0.05	0.46±0.01	4400±314
After incubation											
<i>Rhodotorula mucilaginosa</i>	SDB	811	1.56	0.29	3109	1.45	0.40	971	1.39	0.48	4080
<i>Aspergillus niger</i>	SDB	1689	1.43	0.36	2231	1.50	0.39	328	1.53	0.40	2559
<i>Aspergillus</i> sp.	SDB	2049	1.50	0.34	1871	1.43	0.43	503	1.32	0.49	2374
<i>Penicillium aurantiogriseum</i>	SDB	1262	1.55	0.33	2658	1.43	0.40	1501	1.26	0.44	4159
<i>Penicillium oxalicum</i>	SDB	1362	1.52	0.35	2558	1.44	0.40	460	1.33	0.39	3018
<i>Talaromyces</i> sp.	SDB	2531	1.48	0.36	1389	1.45	0.41	246	1.06	0.44	1635
Mean ± SE		1617±250	1.51±0.02	0.34±0.01	2303±250	1.45±0.01	0.41±0.01	668±196	1.32±0.06	0.44±0.02	2971±406

Table S6 The molecular weight distribution of the typical bacterial and fungal exometabolites.

Species	Low Molecular Weight (LMW) (150 – 300 Da)	Medium Molecular Weight (MMW) (300 – 500 Da)	High Molecular Weight (HMW) (500 – 800 Da)	Total
Bacteria				
<i>Streptomyces pratensis</i>	665 30.8%	1034 47.9%	459 21.3%	2158 100%
<i>Bacillus subtilis</i>	460 31.1%	731 49.4%	288 19.5%	1479 100%
<i>Bacillus toyonensis</i>	540 33.8%	774 48.5%	282 17.7%	1596 100%
<i>Pantoea vagans</i>	617 35.1%	837 47.5%	307 17.4%	1761 100%
<i>Stenotrophomonas</i> sp.	721 25.1%	1244 43.4%	903 31.5%	2868 100%
<i>Pseudomonas baetica</i>	123 18.9%	252 38.7%	276 42.4%	651 100%
Fungi				
<i>Rhodotorula mucilaginosa</i>	318 32.7%	518 53.4%	135 13.9%	971 100%
<i>Aspergillus niger</i>	55 16.8%	155 47.3%	118 35.9%	328 100%
<i>Aspergillus</i> sp.	78 15.5%	299 59.4%	126 25.1%	503 100%
<i>Penicillium aurantiogriseum</i>	187 12.5%	929 61.9%	385 26.6%	1501 100%
<i>Penicillium oxalicum</i>	100 21.7%	222 48.3%	138 30.0%	460 100%
<i>Talaromyces</i> sp.	54 21.9%	164 66.7%	28 11.4%	246 100%

75 **Table S7** The elemental compositions of the typical bacterial and fungal exometabolites.

Species	CHO	CHON	CHOS	CHONS	CHONSP	Total
Bacteria						
<i>Streptomyces pratensis</i>	137 6.4%	1552 71.9%	43 2.0%	426 19.7%	0 0.0%	2158 100%
<i>Bacillus subtilis</i>	150 10.1%	1022 69.1%	37 2.5%	270 18.3%	0 0.0%	1479 100%
<i>Bacillus toyonensis</i>	134 8.4%	1070 67.0%	52 3.3%	340 21.3%	0 0.0%	1596 100%
<i>Pantoea vagans</i>	262 14.9%	1131 64.2%	45 2.6%	318 18.1%	5 0.2%	1761 100%
<i>Stenotrophomonas</i> sp.	140 4.9%	1494 52.1%	69 2.4%	1165 40.6%	0 0.0%	2868 100%
<i>Pseudomonas baetica</i>	25 3.8%	405 62.2%	5 0.8%	216 33.2%	0 0.0%	651 100%
Fungi						
<i>Rhodotorula mucilaginosa</i>	203 20.9%	525 54.1%	58 6.0%	185 19.0%	0 0.0%	971 100%
<i>Aspergillus niger</i>	80 24.4%	233 71.0%	15 4.6%	0 0.0%	0 0.0%	328 100%
<i>Aspergillus</i> sp.	120 23.9%	377 74.9%	5 1.0%	1 0.2%	0 0.0%	503 100%
<i>Penicillium</i>	402	1017	71	10	1	1501
<i>aurantiogriseum</i>	26.8%	67.7%	4.7%	0.7%	0.1%	100%
<i>Penicillium oxalicum</i>	94 20.4%	366 79.6%	0 0.0%	0 0.0%	0 0.0%	460 100%
<i>Talaromyces</i> sp.	182 74.0%	64 26.0%	0 0.0%	0 0.0%	0 0.0%	246 100%

Table S8 The formulas of possible pigments in exometabolites from *Penicillium* and *Talaromyces*.

Genus	Species	Pigment	Formula
<i>Penicillium</i>	<i>Penicillium oxalicum</i>	Carviolin	C ₁₆ H ₁₂ O ₆
		Phoenicin	C ₁₄ H ₁₀ O ₆
	<i>Penicillium aurantiogriseum</i>	Naphthalic anhydride	C ₂₁ H ₂₂ O ₉
		Carviolin	C ₁₆ H ₁₂ O ₆
		Phoenicin	C ₁₄ H ₁₀ O ₆
		Atrovenetin	C ₁₉ H ₁₈ O ₆
		(10Z)-12-carboxymonascorubin (PP-O)	C ₂₃ H ₂₄ O ₇
		N-glutarylribropunctamine	C ₂₆ H ₂₉ NO ₈
		Purpurquinone-A	C ₂₁ H ₂₀ O ₉
		Northerqueinone	C ₁₉ H ₁₈ O ₇
		Xanthomonasin A	C ₂₁ H ₂₄ O ₇
		Trihydroxymethyl-antraquinone	C ₁₅ H ₁₀ O ₅
		Phoenicin	C ₁₄ H ₁₀ O ₆
		Carviolin	C ₁₆ H ₁₂ O ₆
<i>Talaromyces</i>	<i>Talaromyces</i> sp.	Naphthalic anhydride	C ₂₁ H ₂₂ O ₉
		Purpurquinone-A	C ₂₁ H ₂₀ O ₉
		Northerqueinone	C ₁₉ H ₁₈ O ₇
		Mitorubrinol	C ₂₁ H ₁₈ O ₈
		Atrovenetin	C ₁₉ H ₁₈ O ₆