Influence of terrestrial and marine air-mass on the constituents and intermixing of bioaerosols over coastal atmosphere

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Abstract:

Coastal environments provide an ideal setting for investigating the intermixing processes between terrestrial and marine aerosols. In this study, fine particulate matter ($PM_{2.5}$) samples categorized into terrestrial, marine and mixed air masses were collected from a coastal location in Northern China. The chemical and biological constituents, including water-soluble ions (WSIIs), metallic elements, and bacterial and fungal aerosols, were investigated from January to March 2018, encompassing both the winter heating and spring dust seasons. Terrestrial air masses constituted 59.94% of the total air masses throughout the sampling period, with a significant increase during severe haze pollution (up to 90%). These air masses exhibited higher concentration of $PM_{2.5}$ (240 μg/m³) and carried more water-soluble ions and metal elements. The terrestrial air mass also contained a larger number of animal parasites or symbionts, as well as human pathogens from anthropogenic emission, such as *Staphylococcus*, *Deinococcus*, *Sphingomonas*,

Lactobacillus, *Cladosporium* and *Malassezia*. Conversely, a significant quantity of saprophytic bacteria such as hydrocarbon-degrading and gut bacteria from the genera *Comamonas*, *Streptococcus*, *Novosphingobium*, *Aerococcus*, and the saprophytic fungus *Aspergillus* were the most abundant species in the marine air mass samples. The mixed air mass elucidates the intermixing process of terrestrial and marine sources, a result of microorganisms originating from both anthropogenic and terrestrial emissions, which including pathogenic microorganisms from hospitals and sewage treatment plants, and a multitude of soil bacteria. A stronger correlation was noted between microorganisms and continental elements in both terrestrial and mixed air mass samples, specifically K^+, Mg^{2+}, and Ca^{2+} derived from soil dust. Marine air masses exhibited a significant correlation with sea salt ions, specifically Na^+. In the mixed air mass sample, a fusion of marine and terrestrial microorganisms is characterized by alterations in the ratio of pathogenic to saprophytic microorganisms when compared to samples derived from either terrestrial or marine sources. This study on the constituents and amalgamation of bioaerosols over the coastal atmosphere encompassing distinct air masses is crucial for understanding the transport, intermixing processes, and health implications of terrestrial and marine air masses.

1. Introduction

Bioaerosols, encompassing bacteria, fungi, viruses, pollen, and cellular debris, etc., are vital aerosol particles in the atmosphere. Notably, bacterial aerosols which can be either free-floating or attached to airborne particles, typically measure between 0.3 and 10 μm (Shen and Yao, 2023; Zhao et al., 2022). Such characteristics allow for extended residence times and a heightened likelihood of long-distance transport from local to distant regions within the atmospheric circulation (Galbán et al., 2021; Smets et al., 2016). Fungal aerosols are prevalent in Earth's near-surface atmosphere, with their origins being diversely found in water, soil, and plants (Fröhlich-Nowoisky et al., 2009). Furthermore, the presence of pathogenic bacteria and fungi in the atmosphere may lead to significant penetration efficiency within the human respiratory system (Fakunle et al., 2021; Jiang et al., 2022).

Relevant studies have demonstrated that the bacterial and fungal concentration and community structure are significantly influenced by emission sources, atmospheric circulation, meteorological conditions, geographical and topographical factors (Liu et al., 2021; Andrés et al., 2021; Li et al., 2019). The geographical and topographical factors, such as terrestrial and marine environments, exhibit significant differences in bioaerosol sources and pollution characteristics. Studies focusing on continental environments, particularly in large inland cities like Beijing (Zhang et al., 2019; Zhang et al., 2022), Xi'an (Wang et al., 2020), Shanghai (Yao et al., 2019), Jinan (Wei et al., 2020) , and Nanjing (Hu et al., 2020) during severe air pollution, have revealed that continental pollutant emissions significantly contribute to air pollution. Furthermore, transboundary transportation of terrestrial air masses on hazy days plays a crucial role in the progression of regional heavy pollution (Xie et al., 2020). The ocean serves as both a potential source and sink for airborne microorganisms (Archer et al.,

2020; Mayol et al., 2017), exhibiting the intricate interplay between continental communities and their transmission across oceanic regions (Cho and Hwang, 2011; Deleon-Rodriguez et al., 2013). Bioaerosols from the oceans may be influenced by long-distance transport from continental sources, such as plants and human pathogens (Elbert et al., 2007; Sharoni et al., 2015). Studies have shown that the concentration and diversity of bacterial and fungal aerosols from marine environment are typically lower than those derived from continental sources (Cao et al., 2024; Xue et al., 2022; Shi et al., 2022). Xu et al. (2019) undertook a thorough investigation of bacterial abundance in Mt. Tai, China. Their findings indicate that variations in airmass from diverse sources could potentially influence the chemical composition of $PM_{2.5}$, which in turn prompts shifts in bacterial groups. Several studies have examined the impacts of terrestrial and marine air masses on chemical constituents and microbial aerosols (Shi et al., 2022; Aswini and Hegde, 2021; Lang-Yona et al., 2022). Generally, the chemical aerosols can be affected by various sources of air masses, which may include local aerosols or remotely transported aerosols. However, it remains unclear whether different air trajectories contribute to the formation of bacterial communities within these particles. There is a dearth of research focused on the influence of different air masses on the chemical and biological composition in coastal cities.

Marine microbial aerosols can be released from ocean micro-surface or transported from land (Prospero et al., 2005) and settle thousands of kilometers away from their source of release (Mayol et al., 2014), exerting significant impacts on ecological and climate systems. Gong et al (2020a) conducted an examination of the microbial composition along Qingdao's coastlines, revealing a higher proportion of bacteria to total microorganisms from continental sources compared to marine sources. Some marine bacteria persist in aerosols after land transportation, Xu et al (2020b) studied the

diversity of bacterial populations in PM$_{2.5}$ across urban and rural areas of Shanghai, finding that airborne microbial communities over coastal cities are more influenced by long-distance transport than those inland. Air samples taken at high altitude zones in coastal Europe and Japan demonstrated the continental transportation of marine microorganisms (Maki et al., 2014). The introduction of marine bacteria into tropospheric free space may modify the airborne microbial composition in continental regions (Cho and Hwang, 2011; Amato et al., 2007; Maki et al., 2014; Polymenakou et al., 2008; Cáliz et al., 2018). Consequently, it is imperative to ascertain the impact of terrestrial and marine air masses on the composition of atmospheric microorganisms, with a particular focus on the influence of the mixing process between terrestrial and marine air masses on bioaerosols. The coastal aerosols provide the ideal conditions for understanding the mixing processes taking place between natural and anthropogenic air masses from terrestrial and marine.

Weihai, a coastal city situated at the confluence of eastern Asia continent and the Northwest Pacific Ocean, is prone to the impact of marine and terrestrial air masses. Typically, Weihai maintains low pollutant emission with an annual average PM$_{2.5}$ concentration below 35 μg /m^3. However, in winter and spring, regional air pollution intensifies, leading to severe air quality issues. This is due to increased inter-regional transportation, resulting in daily average PM$_{2.5}$ concentration greater than 150 μg/m^3 (Wei et al., 2020). In this study, we conducted an integrated atmospheric observation experiment to examine the potential impact of terrestrial and marine air-mass on the constituents and amalgamation of bioaerosols over the coastal atmosphere. This approach allows for a comprehensive exploration of the effects of sea-land air mass exchange on the spatial and temporal distribution of aerosols, as well as potential intermixing processes in coastal regions.

2. Materials and methods

2.1 Sample collection

The sampling site was situated at the national air sampling station (37.53°N, 122.06°E), 41 m above sea level, and approximately 1-2 km from the coast (Fig. S1). The sampling platform was about 15 m above ground level, and devoid of any significant obstructions. $PM_{2.5}$ samples were gathered between January and March, 2018, during the winter heating and spring dust seasons in northern China. Two parallel $PM_{2.5}$ samplers (TH-150C-III, Wuhan Tianhong Instrument Co., Ltd., China) with a particle size of 2.5 ± 0.2 μm were utilized, equipped with Quartz membrane for the collection of $PM_{2.5}$, inorganic ions, metal elements, and microorganisms at a flow rate of 100 L min^{-1}. The Quartz membranes were cauterized in a muffle furnace at 450°C for 6 hours to remove carbonaceous and contaminant materials. Intermittent sampling was used and samples were collected twice a day (7:00 to 18:30 and 19:00 to 06:30 the following day). During the sampling process, the pre-weighed filter membrane was carefully positioned onto the filter mesh within a clean sampling folder using tweezers. The membrane's rough side should be oriented towards the direction of air intake, and it should be firmly pressed until there is no detectable air leakage. Prior to membrane replacement, the membrane tray is wiped with alcohol cotton and dried. After sampling, the membrane is removed using tweezers. Blank samples were procured by putting the membrane into the sampler head without initiating the sampler. After sampling, the blank samples were extracted simultaneously with the collected sample membranes. All samples were then placed in a sterile filter cassette, encased in clean aluminum foil, and stored at -80°C until further analysis. During the whole sampling period from January 1 to March 31, a total of 102 $PM_{2.5}$ samples over 51 days were obtained, out of which

143 24 samples were chosen for subsequent analysis based on air mass categories.

144 Meteorological parameters, such as air temperature, relative humidity, wind direction,

145 and wind speed, were monitored in situ utilizing a PC-4 automatic weather station (PC-

146 4, JZYG, China). The hourly concentration of $PM_{2.5}$, PM_{10}, CO, SO_2, NO_2, and O_3 were

147 systematically retrieved from the National Ambient Air Quality Monitoring System

148 (http://www.cnemc.cn/).

149 The mass concentration of $PM_{2.5}$, water-soluble ions, and metal elements were

150 quantified after sampling. The membranes were meticulously weighed utilizing a

151 Mettler XP-6 balance with an accuracy of 10^{-6} g. Prior to the weighing, the membranes

152 were maintained in a controlled environment with consistent temperature and humidity

153 for a duration of 24 hours. Ion chromatography (ICS-2100, Chameleon 6. 8, AS-DV

154 autosampler Thermo Fisher) was employed to determine the concentration of water-

155 soluble ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, SO_4^{2-}, and NH_4^+. These ions were

156 extracted by sonication with ionized water for 45 min and then separated by anion

157 (IonPacAS23) or cation column exchange (IonPacCS12A). Then, these ions were

158 detected using a conductivity detector with an anion separation column of IonPacAS23,

159 flow rate: 1.0 mL/min, an anion suppressor of AERS500, and conductivity detector. The

160 injection volume was 25 μL, and the cation separation column was IonPacCS12A, flow

161 rate:1.0 mL/min, cation suppressor was CERS500 (Zhang et al., 2022). Metallic

162 elements including Al, Fe, Ti, Mn, Co, Ni, Cu, Zn, Ga, Sr, Cd, Sn, Sb, Pb, V, Cr, and

163 As, were extracted using microwave digestion extraction (ETHOS ONE, Milestone),

164 with the concentrations determined by ICP-MS or ICP-OES (Thermo Fisher).

165 **2.2 Air mass clustering and classification**

166 The potential sources and transportation of air mass were examined using the

167 MeteoInfo   backward   trajectory   model   (MeteoInfo   3.7.4   –   Java,

168 http://www.meteothink.org/downloads/index.html) developed by the Chinese Academy

169 of Meteorological Sciences. Backward trajectories were simulated at one-hour intervals

170 and estimated over a 24-hour period. The meteorological data were sourced from

171 GDAS1 (ftp://arlftp.arlhq.noaa.gov/pub/archives/gdas1/). Backward trajectories of air

172 masses at an altitude of 500 meters were categorized and clustered, with daily plots

173 illustrating these trajectories. In this study, air mass categories were defined that if more

174 than 90% of the masses originated from the ocean, it was a typical sample affected by

175 marine air-mass; a typical terrestrial air-mass sample if more than 90% originated from

176 the continent; and a mixed air-mass sample if the proportions of terrestrial and marine

177 air masses were similar or accounted for more than 40% of the total air masses in one

178 day.

179 **2.3 DNA extraction and qPCR amplification**

180 Microbial genomic DNA were procured from filters utilizing the Fast-DNA^{TM} SPIN

181 kit for soil (MoBio Laboratories, Carlsbad, CA, USA). The concentration and quality

182 of the extracted DNA was measured via a Nanodrop spectrometer (Nanodrop 2000,

183 Thermo Scientific USA). The DNA concentration in the collected samples ranged from

184 10 to 20 ng/μL, satisfying the prerequisites for subsequent PCR amplification. The

185 quantitative polymerase chain reaction (qPCR) was employed to identify bacterial 16S

186 rRNA and fungal ITS gene copy numbers per cubic meter of air. The bacterial 16S V3-

187 V4 variable region was selected for PCR amplification using primer 338F (5'-

188 ATCTACGGGGGGGCAGCAG-3') and 806R (5'GGACTACHVGGGTWTCTAAT-3')

189 (Masoud et al., 2011). The fungal ITS region were amplified using the primers ITS1

190 (5′-CTTGGTCATTTAGAGGAAGTAA-3′)   and   ITS2   (5′-

191   GCTGCGTTCTTCATCGATGC-3′) (Liu et al., 2021).

192   The PCR amplification conditions comprised an initial denaturation at 95°C

193   for 5 minutes, succeeded by 30 seconds at 95°C, 30 seconds at 50°C, and then 35 cycles

194   at 72°C for 40 seconds, followed by a final extension at 72°C for seven minutes to

195   ensure comprehensive amplification. Fluorescent signals were gathered during the

196   extension phase. For each sample, qPCR was conducted in triplicate, with ultrapure

197   water serving as a negative control to identify potential PCR contamination. Standard

198   curves were constructed using *E. coli* harboring the 16S rRNA gene and *Streptomyces*

199   plasmids containing the ITS gene. Additionally, gradient dilutions of these plasmids

200   were performed, ranging from 10^2-10^7 copies/μL. The copy number of 16S rRNA gene

201   in bacterial genome ranges from 1 to 21 with an average value of 5.5 according to the

202   rrnDB database (https://rrndb.umms.med.umich.edu/) (Stoddard et al., 2015). The

203   number of rRNA copies per genome ranges from 50 to 100 in filamentous fungi

204   (Rooney and Ward, 2005) and *Ascomycetes* typically have relatively smaller genome

205   sizes (Kullman et al., 2005). Consequently, the copy number of the fungal ITS gene in

206   their genome was set at 50 in this study. The FTC-3000 real-time quantitative

207   PCR system (Funglyn Biotech Inc., Scarborough, ON, Canada) was employed for

208   standard curve construction and data processing.

209   **2.4 16S rRNA and ITS gene sequencing and data processing**

210   The V3-V4 region of the bacterial 16S rRNA and the fungal ITS1 gene were targeted

211   for PCR amplification utilizing barcode-specific primers 338F-806R and ITS1F-ITS2,

212   respectively. To ensure optimal amplification efficiency and precision, a high-fidelity

213   enzyme (Phusion® High Fidelity PCR Master Mix from NewEngland Biolabs) in

214   conjunction with GC buffer was employed during PCR amplification.

215   This procedure entailed a pre-denaturation step at 98°C for one minute, followed by 30

216   cycles of 98°C for ten seconds; 58°C for bacteria (56°C for fungi) for 30

217   seconds; and 72°C for an additional 30 seconds, with a final extension at 72°C for five

218   minutes. Following amplification, samples were purified using the Agencourt Ampure

219   XP kit (Beckman Coulter, Brea, CA, USA). Subsequently, the purified samples were

220   combined to achieve equimolar concentrations and analyzed on the Illumina MiSeq

221   PE300 platform (Illumina, Inc. in San Diego, CA).

222   Following sequencing, the barcode sequences were extracted and subsequently

223   stored in fastq format utilizing the QIIME toolkit (Caporaso et al., 2010). Sequences

224   shorter than 200 base pairs, with mass of less than 25, and containing ambiguous bases

225   underwent quality control screening using Trimmomatic (Bolger et al., 2014) and

226   Mothur (Schloss et al., 2009). The assembled sequences were then de-duplicated and

227   trimmed to equal length. De-chimerized sequences were eliminated (Edgar, 2013), and

228   sequences were clustered into operational taxonomic units (OTUs) using UPARSE at a

229   97% identity threshold. Subsequently, individual OTUs were removed. Taxonomic

230   assignments were determined using a Basic Local Comparison Search Tool (BLAST)

231   search against the bacterial SILVA taxonomy release 138.2 dataset and fungal UNITE

232   ITS reference v8.0 dataset. The original raw sequences were deposited in the Sequence

233   Read Archive under the accession number PRJNA1101427 and PRJNA1101176.

234   **2.5 Statistical analysis**

235   Bacterial community functional analysis was conducted using FAPROTAX, a

236   manually constructed database that maps prokaryotic taxa to metabolic or other

237   ecological functions, such as sulfur, nitrogen, hydrogen, and carbon cycling (Chen et

238   al., 2022). FUNGuild (Fungi Functional Guild) was used to predict the fungal

239 ecological function. This tool could classify and analyze fungal communities by the

240 microecological guild based on current published literature or data from authoritative

241 websites to classify fungi functionally (Nguyen et al., 2016). Three primary groups are

242 obtained based on the nutritional mode: Pathotroph, Symbiotroph, and Saprotroph.

243 Samples affected by different air masses were examined for intergroup species

244 variability, based on community abundance data. This was achieved using rigorous

245 statistical methods to identify species demonstrating differences in abundance within

246 the microbial communities of different groups, and hypothesis testing to evaluate the

247 significance of these observed differences. Statistical analysis, including Analysis of

248 variance (ANOVA) and Kruskal Wallis tests, were employed to discern bacteria and

249 fungi with varying abundances between samples and groups. A p value less than 0.05 or

250 0.01 was considered significant. ANOVA was used to analyze variation in a response

251 variable measured under conditions defined by discrete factors (Martin G. Larson,

252 2008). The Kruskal-Wallis test determines whether there is a statistically significant

253 difference between the medians of three or more independent groups (Kassambara,

254 2019). The Mantel analysis was utilized to reveal the correlation between microbial

255 community composition and various environmental factors. The Pearson's r coefficient

256 at $p < 0.05$ and $p < 0.01$ indicates the significant correlation.

257 **3. Results**

258 **3.1 Air masses categorization and typical pollution processes**

259 The Meteolnfo backward trajectory model was employed to simulate the trajectories

260 of air masses at an altitude of 500 m in the coastal city over 24-hour period,

261 identifying and classifying potential sources of air mass transport (Fig. S1). The

262 terrestrial air mass accounted for 59.94% of the total air masses at the sampling

11

263   site throughout the period, exhibiting an average $PM_{2.5}$ concentration of 36.15±26.52

264   μg/m³. Severe air pollution episodes occurred on January 20, with $PM_{2.5}$ was the

265   primary pollutant and the concentration reaching 240 μg/m³. During regional haze

266   pollution, the terrestrial air masses primarily influenced the coastal city were typically

267   originating from the North China Plain and the surrounding areas. The primary

268   contributors to emissions in the terrestrial air mass of this region are the dense

269   population, industrial and agricultural activities. The impact of the spring dust is mainly

270   reflected in the primary pollutant PM_{10} from March 30 to March 31. A significant

271   increase in PM_{10} concentration was observed on March 31, with an hourly maximum

272   value of 167 μg/m³. A relatively low $PM_{2.5}/PM_{10}$ ratio of 0.25 indicated pronounced

273   dust pollution. The typical marine air masses mainly from the eastern and southern areas

274   from the Yellow Sea and the western Pacific edge. Marine air masses from northern

275   areas partly come from the Bohai Sea, crossing inland areas and possibly making

276   contact with the land prior to reaching the study area (Fig. S1). A total of 14% of the

277   sampling days were influenced by marine air masses, resulting in an average $PM_{2.5}$

278   concentration of 23.99±11.00 μg/m³. Mixed air masses, characterized by simultaneous

279   influence from the northwestern winds of Inner Mongolia and the offshore air masses

280   of the Bohai Sea or Yellow Sea, accounted for 27% of all affected sampling days. These

281   mixed air masses yielded an average $PM_{2.5}$ concentration of 45.11±12.69 μg/m³. In

282   comparison, under the influence of mixed air masses, pollutant concentrations were

283   notably high. During the spring dust season, there is a notable increase in the proportion

284   of mixed air mass. This elevated concentration of particulate matter correlates with the

285   transmission of sand dust (Xie et al., 2020).

286   Three heavy pollution events were investigated to explore the alterations in air mass

287   during these episodes (Fig. 1, Fig. S2). Generally, the initiation and development stages

of pollution events were predominantly characterized by terrestrial and mixed air

masses. Cold northwestern airmass from the continent, and marine air masses from east

or south, were the primary contributors during pollution mitigation. For pollution

episode I occurred from January 19 to January 22, the western terrestrial air mass was

predominantly responsible for initiating the pollution, evidenced by a $PM_{2.5}$

concentration of 51.35 μg/m^3. As the pollution progressed, it transitioned into a mixed

air mass with a $PM_{2.5}$ concentration peaking at 240 μg /m^3. This change was

accompanied by significant increases in WSIIs and elemental concentrations. Pollution

elimination was initiated with high winds, snowfall influenced by the cold northwestern

airmass from the continent and marine air masses from the northeast sea. The average

$PM_{2.5}$ concentration was reduced to 7.92 μg /m^3, accompanied by a decrease in both

WSIIs and elemental concentrations.
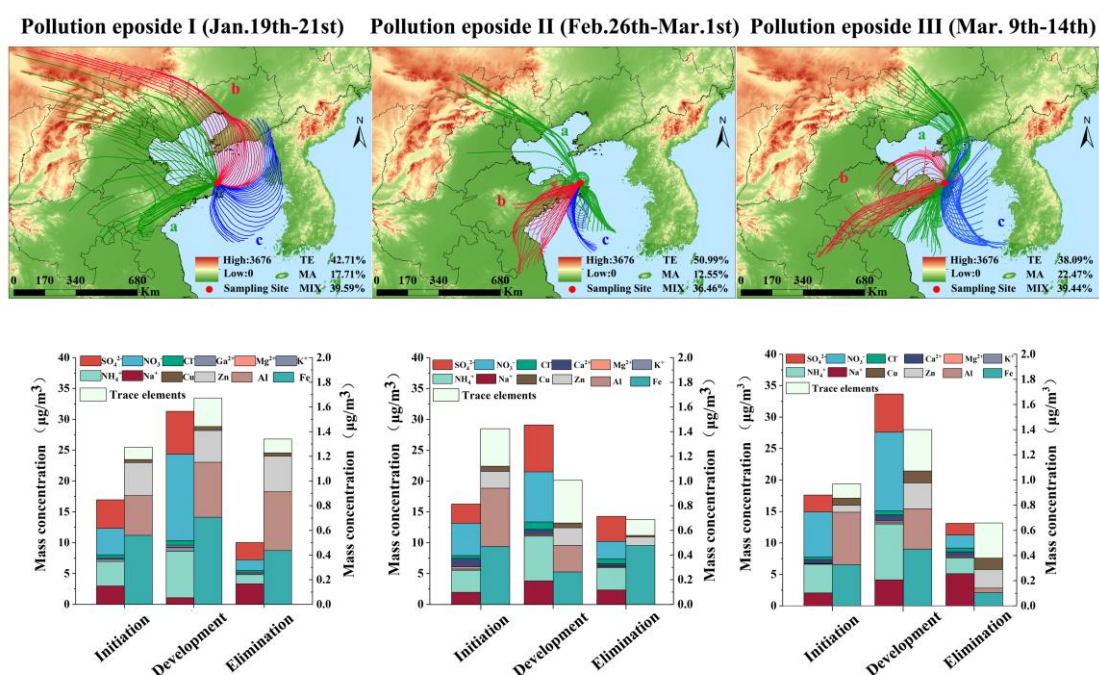
Fig. 1 Transformation of terrestrial and marine air masses, and chemical composition in

$PM_{2.5}$ of three severe air pollution episodes. a, pollution initiation; b, pollution development;

c, pollution elimination. TE, terrestrial air mass; MA, marine air mass; MIX, mixed air

mass.

3.2 Water-soluble ions and metal elements over coastal atmosphere

During the sampling period, the concentration of water-soluble ions in $PM_{2.5}$ was 22.92 ± 12.19 μg/m^3, ranging from 4.58 to 78.14 μg/m^3. In marine air-mass samples, a lower concentration of water-soluble ions was observed, with the concentration of 13.01 ± 7.43 μg/m^3, 27.94 ± 13.61 μg/m^3 and 30.38 ± 11.38 μg/m^3 in marine, terrestrial and mixed air masses, respectively. Notably, NO_3^- had the highest proportion (26.94%, 6.4%~52.6%), followed by SO_4^{2-} (21.94%, 9.4%~33.4%) and NH_4^+ (20.26%, 5.8%~35.6%). The SO_4^{2-}, NO_3^- and NH_4^+, constituted the majority of total ions in other studies conducted in Beijing, Shanghai, and Guangzhou (Pathak et al., 2008; Hu et al., 2014; Zhou et al., 2012). These inorganic secondary ions were significantly influenced by both terrestrial and mixed air masses, with the latter exhibiting a more pronounced effect (NO_3^-, 10.65 ± 3.26 μg/m^3; NH_4^+, 7.39 ± 3.30 μg/m^3; SO_4^{2-} 6.76 ± 1.77 μg/m^3) (Fig. S3).

A high concentration of Na^+ was observed, with a range of 3.15 ± 1.69 μg/m^3, and accounted for 14.47% of the total water-soluble ions. The concentration of Na^+ and Mg^{2+} exhibited analogous trends across terrestrial, marine, and mixed air mass samples. The ratio of Mg^{2+}/Na^+, typical components of sea salt, was determined to be 0.11. This value closely corresponded to the 0.12 found in seawater, indicating a potential origin from marine environments (Sun et al., 2022). The concentration of K^+ was 0.24 ± 0.20 μg/m^3 and 0.26 ± 0.10 μg/m^3 in the terrestrial and mixed air-mass samples, and was twice as high as those in the marine air-mass samples (0.11 ± 0.05 μg/m^3), which suggested an important contribution from anthropogenic emissions. The concentration of Cl^-, and Ca^{2+} was high in terrestrial and mixed air mass samples. Generally, these two ions mostly come from anthropogenic or terrestrial environments, such as Cl^- mainly comes from sea salt, coal and biomass combustion, and Ca^{2+} is a crustal element associated

with soil dust and sandstorms (Liang et al., 2021). Overall, from the composition and concentration of water-soluble ions in $PM_{2.5}$, the coastal city was more affected by sea salt, coal combustion, and dust events in early spring.

The elements concentration in different air-mass samples are depicted in Fig. S3. The top ten elements were found to be Fe, Al, Zn, Cu, Sn, Pb, Mn, Ti, Ni, and V. These metal elements were categorized into macro and trace elements. The macro metals, specifically Iron (Fe), Aluminum (Al), and Zinc (Zn), constituted a significant proportion of the total heavy metal elements, accounting for 34%, 25%, and 23% respectively. In general, the concentration of both macro and trace metal elements in marine air masses was found to be lower than that in terrestrial and mixed air masses. The V/Ni ratio in marine air-mass samples was found to be 0.78, a value significantly higher than that of both terrestrial and mixed air masses. The V/Ni ratio serves as an indicator to assess the influence of ship emissions. A ratio exceeding 0.7 typically indicates a significant impact from ship emission sources, and is commonly used as an indicator in coastal cities (Zhang et al., 2014).

**3.3 Microbial community over coastal atmosphere**

Airborne bacterial and fungal concentration in $PM_{2.5}$ were $3.29\pm2.38\times10^6$ copies/m^3 ($1.99\pm1.44\times10^6$ bacteria m^{-3}, $P=0.06$) and $1.69\pm0.55\times10^6$ copies m^{-3} ($3.39\pm1.10\times10^4$ fungal spores m^{-3}, $P=0.04$). This value was comparable to the average abundance of bacteria and fungi in the atmospheric boundary layer over land, which is approximately 1.9×10^4 bacteria m^{-3} and 2.4×10^4 fungal spores m^{-3} (Mayol et al., 2014). A high concentration was observed in terrestrial air-mass samples, with the average value of $2.59\pm2.16\times10^6$ copies m^{-3} ($P=0.12$) and $3.18\pm0.85\times10^6$ copies m^{-3} ($P=0.007$) for bacteria and fungi, respectively. Microbial concentration in marine air-mass samples

354 was significantly lower, with the average concentrations of bacteria and fungi being

355 $2.70\pm1.00\times10^5$ copies m^{-3} (P=0.04) and $3.08\pm1.55\times10^5$ copies m^{-3} (P=0.07).

356   Airborne microbial community structure exhibited significant variation influenced

357 by marine and terrestrial air masses (Fig. 2). Predominantly, *Proteobacteria* (40.06%),

358 *Firmicutes* (36.30%), *Actinobacteria* (8.97%), *Bacteroidetes* (8.29%), and

359 *Deinococcus-Thermus* (4.59%) were identified as the most abundant bacteria. Notably,

360 *Actinobacteria* and *Deinococcus-Thermus* were found in high concentration in samples

361 from terrestrial air masses. In particular, *Deinococcus-Thermus* demonstrated a relative

362 abundance of 7.9% in terrestrial air mass samples, significantly surpassing that of

363 marine (1.2%) and mixed air mass samples (1.5%). *Cyanobacteria* exhibit a higher

364 concentration in both terrestrial and mixed air-masses samples. *Cyanobacteria* are

365 prevalent in freshwater, soil, surface crusts in deserts (Cordeiro et al., 2020; Temraleeva

366 et al., 2016; Curren and Leong, 2020). In comparison to terrestrial environments, the

367 prevalence of *Cyanobacteria* is notably diminished in colder marine environments

368 (Koh et al., 2012). Atmospheric *Cyanobacteria* in the coastal city may originate from

369 the terrestrial environment, subsequently transported via terrestrial and mixed air

370 masses. *Proteobacteria* was the most prevalent taxon in marine air-mass samples. The

371 predominant metabolic activity in deep-sea sediments is attributed to this group, with

372 major taxa being found in marine sediments (Huang et al., 2021). The predominant

373 bacterial genera included *Staphylococcus* (21.02%), *Enhydrobacter* (6.43%),

374 *Lactobacillus* (6.03%), and *Deinococcus* (4.56%). These bacteria included a series of

375 opportunistic pathogens and were found abundant in terrestrial and mixed air masses

376 samples. Relative abundance of bacteria such as *Comamonas*, *Streptococcus*,

377 *Ruminococcus*, and *Enterobacteriaceae* were higher in marine air-mass samples. These

378 predominantly consisted of saprophytic bacteria and intestinal microorganisms.

379   The dominant fungal phyla were *Ascomycota* (77.29%) and *Basidiomycota* (21.58%),

380   which were similar to the previous studies (Du et al., 2018; Liu et al., 2019; Zeng et al.,

381   2019). Fungal community influenced by terrestrial and mixed air masses were quite

382   similar, with relatively higher abundances of opportunistic pathogens such as

383   *Malassezia*, *Alternaria*, *Cladosporium*. In contrast the saprophytic *Aspergillus*,

384   *Davidiellaceae* and *Coprinellus* were abundant in marine air-mass samples.
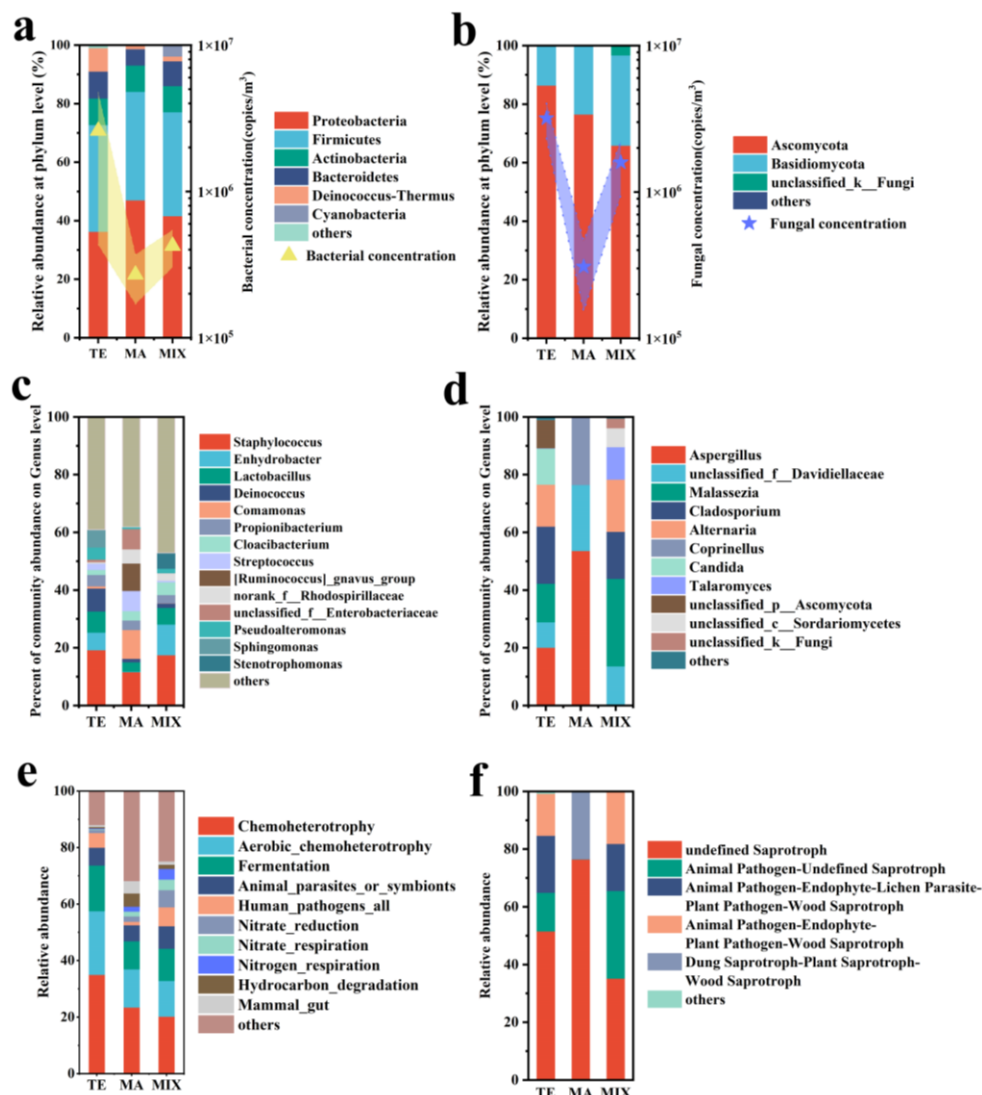
385

386   **Fig. 2. Bacterial and fungal community composition and function influenced by different air**

387   **masses. The community concentration, main phylum (a), (b), genus (c), (d), and community**

388   **function (e), (f) are indicated.**

389   The FAPROTAX analysis revealed that the primary bacterial ecological functions

390   were chemoheterotrophy, aerobic chemoheterotrophy, fermentation, and human

pathogens. These accounted for 74%, 47%, and 44% respectively in terrestrial, marine, and mixed air-mass samples. Notably, human pathogens and animal parasites or symbionts were more prevalent in terrestrial and mixed air-mass samples than in marine air-mass samples. Marine air-mass samples were enriched with mammal gut bacteria, as well as hydrocarbon and aromatic compound degradation bacteria. The fungal community function in terrestrial and mixed air-mass samples were similar, with undefined Saprotroph, Animal Pathogen-Undefined Saprotroph, Animal Pathogen-Endophyte-Lichen Parasite, Animal Pathogen-Endophyte-Plant Pathogen were the main functions, which totaled 99.27%, 99.98%, 99.27% in the terrestrial, marine, and mixed air-mass samples. In particular, the observed prevalence of Saprotroph fungi was higher in samples from marine air masses, such as those containing *Aspergillus*. Notably, the fungi associated with terrestrial air masses predominantly carried animal pathogens and exhibited greater pathogenicity, including species such as *Malassezia* and *Alternaria*.

4. Discussion

4.1 Community disparities influenced by terrestrial and marine air masses

Overall, bacterial and fungal community concentration influenced by terrestrial and mixed air masses were higher than those in marine air mass samples. Bioaerosols originating from anthropogenic environments, such as sewage treatment plants and hospitals, may harbor more resistant bacteria. Microorganisms from arid areas like deserts possess a higher number of species adapted to harsh conditions, including resistance to temperature fluctuations, dryness, and UV radiation. These include bacteria such as *Bacillus*, *Streptococcus*, *and Deinococcus* (Maki et al., 2010; Park et al., 2018; Qi et al., 2021). These microorganisms can adhere to particulate matter during

415   long-distance transport of air masses after being released from their terrestrial habitats.
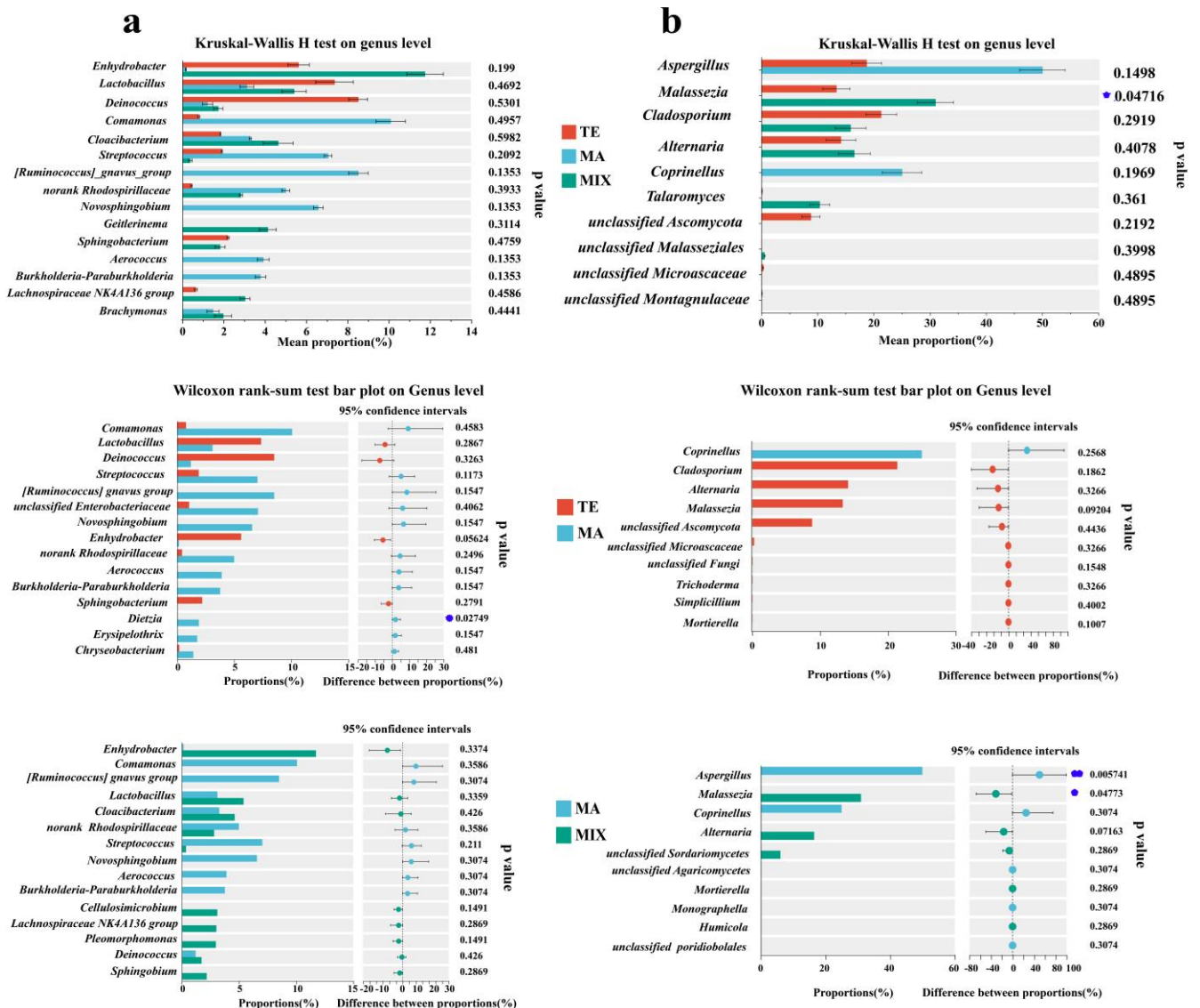
Fig. 3 Bacterial (a) and fungal (b) community disparities influenced by terrestrial (n=10), marine (n=6), and mixed air mass (n=8).
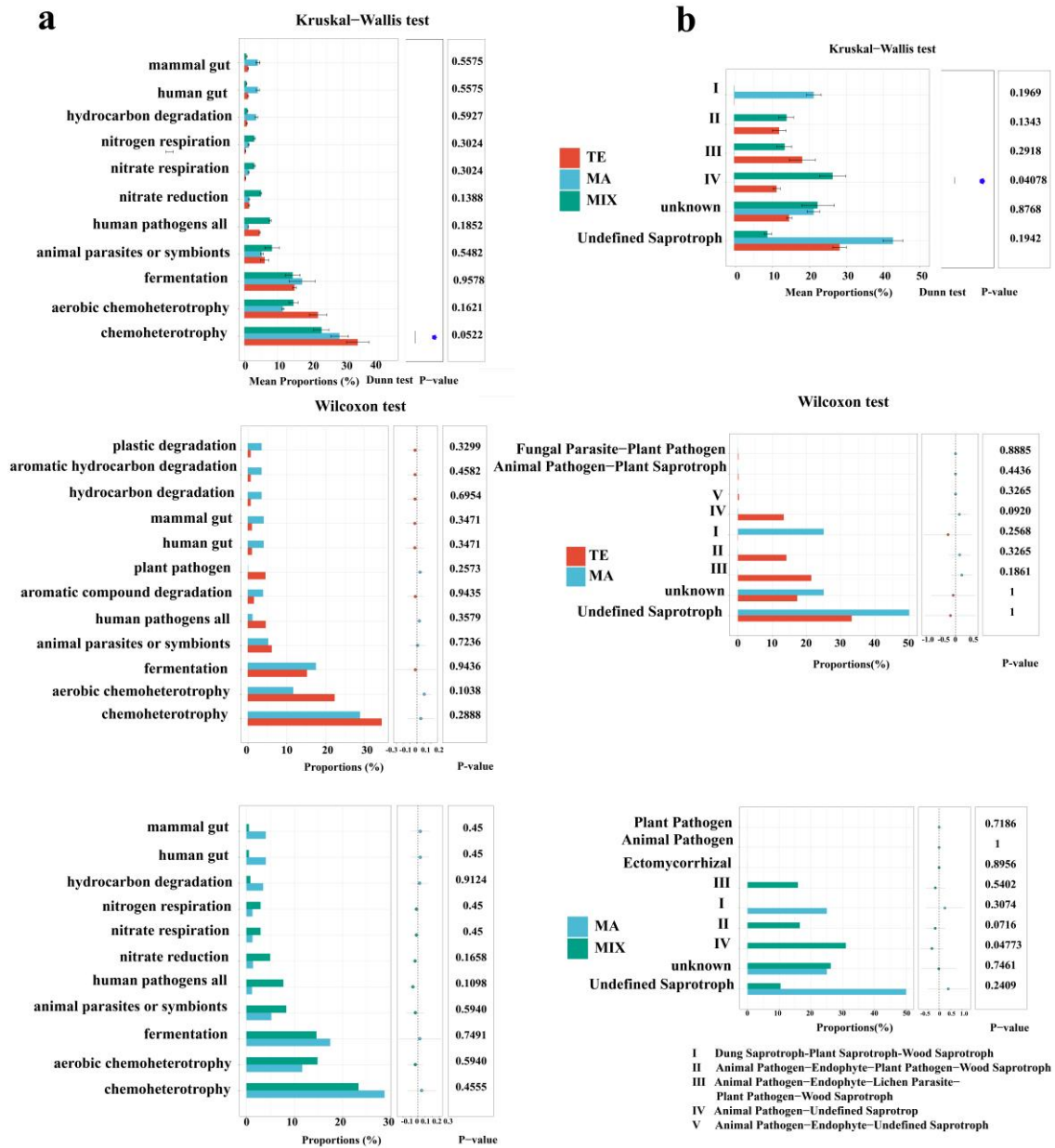

420   Community disparities influenced by terrestrial, marine, and mixed air masses was

421   shown in Table S2, S3, Fig. 3 and Fig. 4. The Principal Coordinates Analysis (PCoA)

422   revealed distinct clusters corresponding to terrestrial, marine, and mixed air mass

423   samples (Fig. S4). In terms of bacterial community variations, numerous dominant

424   microorganisms exhibited obvious differences in abundance between marine and

425   terrestrial air mass samples, although these differences were not statistically significant

426   (p>0.05). For instance, the bacterial enrichments in terrestrial and mixed air-masses

included *Staphylococcus*, *Deinococcus*, *Lactobacillus*, and *Sphingomonas*. *Staphylococcus is* a pathogenic bacterium widely found in human skin and intestines that can cause various infections (Cheung et al., 2021). As a radiation-resistant bacterium, *Deinococcus-Thermus* is capable of withstanding harsh environmental conditions (Callegan et al., 2008; Rainey et al., 2007). *Lactobacillus*, a genus of Gram-positive bacteria, has also been identified as abundant in atmospheric dust (Federici et al., 2018; Xu et al., 2017). *Sphingomonas*, typically found in water and soil, which is widely known as the metabolic mechanism ability to endure poor nutrients and degradation of refractory compounds, such as polycyclic aromatic hydrocarbons (PAHs) (Hu et al., 2007; Sun et al., 2018). The majority of these bacteria possess the ability to produce spores, which are capable of withstanding harsh conditions such as low temperatures, aridity, and radiation during long-distance transmission, thereby ensuring their survival throughout this process (Griffin et al., 2003). *Comamonas* was identified as the dominant bacterium in the coastal atmosphere of Weihai, particularly prevalent in marine air-mass samples. This bacterium is commonly associated with environmental bioremediation and is predominant in oligotrophic environments (Yan et al., 2012; Zhang et al., 2024). *Streptococcus* are mostly found in the oral and gastrointestinal tracts and also play an important role in human infections (Brouwer et al., 2023). *Ruminococci*, and *Enterobacteriaceae* are typical gut microorganisms present in humans and animals. In the coastal atmosphere, the identified sequences of these gut bacteria might be associated with intestinal microorganisms in marine fish and mammal animals.

For fungal community, *Aspergillus* demonstrated a significant differentiation between marine and mixed air mass samples (P=0.005). The highest proportion was noted in samples from marine air masses, at 53.7%. In contrast, the values were 20.1%

and 0.3% in terrestrial and mixed air masses respectively. Previous studies have confirmed that *Aspergillus* is widely distributed in nature and unpolluted environments (Kendrick, 1995). This fungus is predominantly found in offshore regions, such as Qingdao, China (Li et al., 2011). Furthermore, the saprophytic *Aspergillus* was also prevalent in clean samples during periods of haze pollution and was frequently detected on non-haze days (Yan et al., 2016). *Malassezia* was higher in terrestrial and mixed air-mass samples ($P=0.047$), which has been found to be widespread in a variety of animals. As a parasitic fungus, *Malassezia* causes the majority of skin diseases, such as dandruff and seborrheic dermatitis caused by *Malassezia sphericalis* (Deangelis et al., 2007). Furthermore, *Cladosporium* exhibits higher abundance in terrestrial and mixed air-mass samples compared to marine air masses, although this difference lacks statistical significance ($P>0.05$). This species is ubiquitous worldwide, commonly found in a wide variety of plants, known to be a common endophyte as well as a foliar fungus (El-Morsy, 2000; Islam and Hasin, 2000). It is frequently isolated from soils, foodstuffs and organic matters (Bensch et al., 2012). Moreover, *Cladosporium* have been commonly observed in terrestrial atmospheric environments, demonstrating the potential origins from continental environments (Frączek et al., 2017; Han et al., 2019).

The significant variance in the ecological function of microbial community was predominantly evident in the Animal Pathogen-Undefined Saprotrophs (*P=0.04*). These pathogenic fungi were more prevalent in terrestrial and mixed air-mass samples than in marine air-mass samples. Marine air-mass samples were enriched with mammal gut bacteria, hydrocarbon and aromatic compound degradation bacteria, and undefined Saprotroph fungi.

475

Fig. 4. Bacterial (a) and fungal (b) community function disparities influenced by
terrestrial (n=10), marine (n=6), and mixed mass (n=8).

476
477

4.2 Implications of environmental factors on coastal airborne microbes

To elucidate the contributions of marine and terrestrial air masses to microbial aerosols in the coastal city, a Mantel correlation analysis was conducted between microbial aerosols and various environmental factors (Fig.5, Fig.S4). The bacterial community exhibited a significant positive correlation with SO_4^{2-} due to the influence of terrestrial and mixed air masses ($P<0.05$). Both bacterial and fungal communities demonstrated positive correlations with continental ions such as K^+, Mg^{2+}, and Ca^{2+} (Shi et al., 2022). Additionally, a positive correlation was observed between bacterial and fungal concentrations and NO_2 ($P<0.05$), as well as a significant positive correlation with PM_{10} ($P<0.01$). Air masses transported over long distances from the continent appear to harbor diverse and abundant microbial populations (Kakikawa et al., 2009; Deleon-Rodriguez et al., 2013). For instance, the long-distance transportation of dust particles from northwestern winds in the Inner Mongolia region may have altered the community structure and abundance (Qi et al., 2021; Xia et al., 2024). Dust-borne bacteria, such as *Staphylococcus*, *Delftia*, *Pseudoalteromonas* and *Deinococcus*, are likely introduced into the atmosphere during Asian Dust events. Most of these bacteria accompany the transportation of dust particles to the downwind coastal city. Similarly, microbial communities showed high positive correlation with ions from continental sources, such as K^+, Mg^{2+}, and Ca^{2+} in terrestrial and mixed air mass samples. K^+ is the signature ion of biomass combustion (Mason et al., 2016; Yu et al., 2018). Mg^{2+} and Ca^{2+} are mostly derived from crustal elements (Zhang et al., 2012). The dominant bacteria within the microbial community exhibited a significant positive correlation with these three ions simultaneously, suggesting a similar sources. They generally originate from terrestrial sources, including anthropogenic biomass combustion and soil dust.
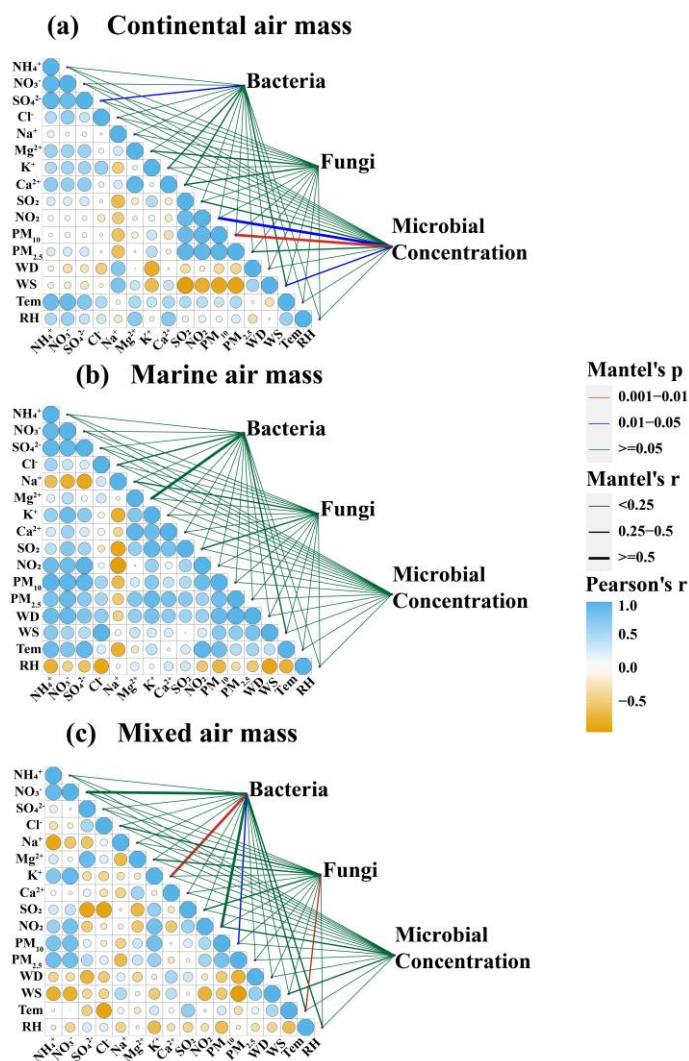
Fig.5 Mantel analysis reveal the correlation between microbial community and various environmental factors under different air masses, terrestrial air masses (a) marine air masses (b) and mixed air masses (c). The Pearson's r coefficient indicates the significant correlation at p<0.05 and p<0.01.

The concentrations of particulate matter and water-soluble ions in samples affected by the marine air mass are notably low, indicating that the majority of chemical elements did not significantly influence the composition and abundance of the microbial community. However, a significant positive correlation was observed between Na^+ and certain dominant species such as *Talaromyces*, *Monographella*, and *Phoma* ($P < 0.05$). Similarly, Mg^{2+} was found to be significantly positively correlated with *Malassezia* in mixed air masses ($P<0.01$) (Fig. S5). Besides anthropogenic sources,

516   such as industrial emissions, the origins of Mg^{2+} and Na^+ in coastal regions should also

517   consider the impact of sea salt (Sun et al., 2022). Samples collected under the influence

518   of marine air masses had a positive correlation between Mg^{2+} and Na^+ (r^2 = 0.67),

519   indicating that they have a similar origin of sea salt. Furthermore, the mean Mg^{2+}/Na^+

520   ratio was found to be 0.11, which is proximate to the seawater value of 0.12 (Seinfeld

521   and Pandis, 1997).

522   Influenced by mixed air masses, temperature has a greater impact on fungal

523   community, which was positively correlated with *Malasseziales* and *Davidiellaceae*. In

524   marine air mass samples, a positive correlation between air temperature and certain

525   microorganisms (*Aerococcus*, *Cloacibacterium*, *Sphingobium*, *Enhydrobacterium*,

526   *Davidiellaceae*, *Malasseziales*) also indicated that the increase in air temperature in

527   spring favors the survival of airborne microbes (Jones and Harrison, 2004).

528   **4.3 Intermixing of bioaerosols from terrestrial and marine air-mass**

529   Coastal atmospheres exhibit a complex amalgamation of terrestrial and marine

530   aerosols, with the characteristics significantly influenced by the origins of the air

531   masses. Consequently, these coastal aerosols offer ideal conditions for examining the

532   mixing processes between natural and anthropogenic emission from terrestrial and

533   marine sources, respectively. This work shows that enormous levels of haze aerosols

534   from the terrestrial and mixed air mass can be rapidly transported into the coastal city

535   during regional haze or dust pollution. During the long-distance transportation of air

536   masses, a comprehensive mixture of soil-derived, biogenic, and anthropogenic

537   microorganisms from terrestrial air masses, and aquatic, saprophytic, gut

538   microorganisms from marine environments are fully mixed (Fig. 6). This amalgamation

539   is particularly evident in the samples of mixed air masses. Within these, a significant

540   quantity of chemical components, bacteria and fungi, as well as opportunistic pathogens,

541   were transported into the coastal city from the continent. Microbial communities were

542   strongly correlated with haze aerosols, e.g., WSIIs in $PM_{2.5}$ from terrestrial and mixed

543   air mass. The primary influence on terrestrial air mass was anthropogenic emissions,

544   with coal combustion for winter heating and biomass burning being the predominant

545   pollution sources. Moreover, dust events in spring carried higher concentration of

546   particulate matter. These air pollutants can act as initial source of bioaerosols such as

547   bacteria and fungi, thereby providing a site for attachment reproduction (Jiang et al.,

548   2022). Additionally, water-soluble ions in PM, primarily secondary inorganic ions, such

549   as sulfate, nitrate, and ammonium ions, can supply essential nutrients for microbial

550   growth (Fan et al., 2019). This elucidates the elevated concentration of particulate

551   matter and microorganisms in terrestrial air mass during heavy pollution.

552   Simultaneously, it is pertinent to highlight that during such pollution events, the

553   terrestrial air mass exacerbates the pollution process, leading to a marked increase in

554   the proportion of pathogenic microorganisms.
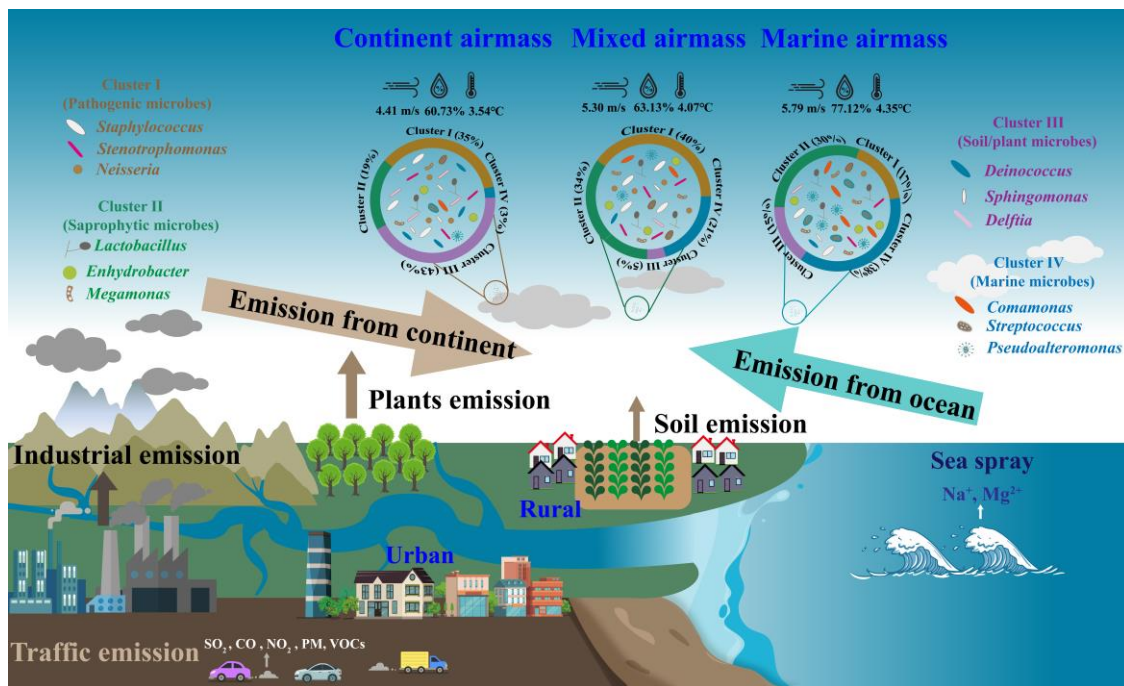
Fig. 6 Implications of intermixing of bioaerosols from terrestrial and marine air-mass on the composition of airborne microbial communities.

In the coastal city of Weihai, marine air masses predominantly originate from the expansive western Pacific Ocean, located to the eastern and southern areas. These air masses are typically characterized by their cleanliness and contain a significant quantity of saprophytic and intestinal microorganisms that infiltrate the atmosphere via ocean droplets (Mayol et al., 2014). The presence of microorganisms in marine air masses exhibited a significant correlation with sea salt ions, specifically Na^+ and Mg^{2+}. These ions, when introduced into the atmosphere, form aerosolized particulate matter. The introduction of marine bacteria into tropospheric free space may alter the airborne microbial composition in continental regions (Cho and Hwang, 2011; Amato et al., 2007; Maki et al., 2014; Polymenakou et al., 2008; Cáliz et al., 2018), thereby significantly impacting bioaerosols over coastal atmospheres.

Notably, marine air masses from the northerly areas, cross China's inland Bohai Sea and then pass through urban and rural areas with high human activity intensity. These areas have more anthropogenic microorganisms, including pathogenic microorganisms

573 from hospitals and sewage treatment plants. In this study, samples within this category

574 are defined as those influenced by mixed air mass. This also signifies the amalgamation

575 of marine and terrestrial microorganisms. Consequently, within the mixed air mass

576 samples, the prevalence of pathogenic and saprophytic microorganisms is comparable.

577 This similarity underscores the complex impact of both terrestrial and marine sources.

578 Populations in coastal cities may also be susceptible to exposure to these bioaerosols

579 and pathogens, which are transported over long distances during regional air pollution.

580 **5. Conclusion**

581   This study evaluated the potential influence and the mixing effects between natural

582 and anthropogenic aerosols respectively from terrestrial and marine air mass on

583 biological constituents of $PM_{2.5}$ over coastal atmosphere. The concentration of water-

584 soluble ions, metal elements and bioaerosols were higher in terrestrial and mixed air-

585 mass samples than marine air-mass samples. The V/Ni ration greater than 0.7 in marine

586 air-mass samples indicated significant influence from marine ship emissions. Bacterial

587 and fungal community influenced by terrestrial and mixed air masses were enriched in

588 pathogens, including the *Staphylococcus*, *Malassezia* and *Alternaria.* In contrast,

589 marine air mass carried more gut and saprophytic microbes, such as *Ruminococcus*,

590 *Enterobacteriaceae*, *Aspergillus,* and *Davidiellaceae*. Potential implications of

591 environmental factors on airborne microbes based on Mantel correlation analysis

592 suggested that the bacterial community was mainly significantly correlated with SO_4^{2-},

593 K^+, Mg^{2+}, and Ca^{2+} and PM_{10} influenced by terrestrial and mixed air masses from

594 continental sources. In contrast, influenced by marine air masses, bacterial and fungal

595 community were strongly correlated with sea salt ions, such as Na^+. The predominant

596 bacteria and fungi in marine and mixed air-mass samples exhibited a positive

correlation with air temperature in spring, suggesting elevated temperature is conducive to the survival and reproduction of bioaerosols. Our research offers a novel perspective on the amalgamation of terrestrial and marine aerosols in the coastal atmosphere. In the mixed air-mass samples, a complex amalgamation of soil-derived and anthropogenic microorganisms from terrestrial air masses, along with aquatic, saprophytic, and gut microorganisms from marine environments, are fully intermixed during the long-distance transportation of air masses. This process significantly influences bioaerosols in coastal atmospheres. This understanding will enhance our comprehension of the environmental and climatic impacts on microbial aerosols within the marine boundary layer. Future research endeavors will concentrate on the local emission and long-distance transport, as well as the contributions of both terrestrial and marine air masses on bioaerosol components in coastal cities.

CRediT authorship contribution statement

All authors contributed to the manuscript and have given approval of the final version. Min Wei designed the study. Qun He performed the data analysis and wrote the original manuscript. Min Wei assisted with the sampling, Qun He, Zhaowen Wang and Rongbao Duan conducted the experiments and performed the statistical analyses. Houfeng Liu, Min Wei, and Pengju Xu contributed to the interpretation of results. Min Wei, Caihong Xu and Jianmin Chen revised the manuscript.

Data availability

The hourly concentration of $PM_{2.5}$, PM_{10}, CO, SO_2, NO_2, and O_3 were systematically retrieved from the National Ambient Air Quality Monitoring System (http://www.cnemc.cn/); the historical air quality data was sourced from the nearest

environmental monitoring center (https://www.aqistudy.cn/historydata/); MeteoInfo

backward trajectory model (MeteoInfo 3.7.4 - Java, http://www.meteothink.org

/downloads/index.html); meteorological data were obtained from GDAS1

(ftp://arlftp.arlhq.noaa.gov/pub/archives/gdas1/), and meteorological data as well as

bacterial and fungal concentrations are available from the authors on request

(minwei@sdnu.edu.cn). The raw sequences of bacterial 16S rRNA and fungal ITS gene

are available in the Sequence Read Archive (SRA) under accession number

PRJNA1101427

(https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA1101427&o=acc_s%3Aa)

and   PRJNA1101176

(https://dataview.ncbi.nlm.nih.gov/object/PRJNA1101176?reviewer=mu6a68nm61vn

h0g1lahff3qvma).

Declaration of competing interest

The authors declare that they have no conflict of interests.

Acknowledgments

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