



Airborne bacterial community associated with fine particulate matter (PM_{2.5}) under different air quality indices in Temuco city, southern Chile

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Abstract

Temuco (Chile) is one of the most polluted cities in Chile and Latin America. Although the fine fraction of particulate matter (PM_{2.5}) has been extensively studied and monitored due to its negative impact on public health, its microbiological components remain unknown. We explored the airborne bacterial community in PM_{2.5} under good, moderate, alert, pre-emergency and emergency indices of air quality (AQIs) established by the Chilean government. Bacterial community relationship with environmental factors (PM_{2.5}, PM₁₀, carbon monoxide, among others), was also evaluated. Significant differences in PM_{2.5} bacterial community composition associated with AQIs were revealed, using 16S rRNA target sequences of denaturing gradient gel electrophoresis (DGGE) bands. Bacterial communities in PM_{2.5} were mainly clustered (80%) into emergency and pre-emergency samples. The dominant phylum was Proteobacteria and most abundant genus was *Novosphingobium*, traditionally related to opportunistic respiratory diseases. The main factors associated with community structure were PM_{2.5}, PM₁₀ and carbon monoxide concentrations. This study exposed that bacterial community composition in Temuco varies according to AQIs, with the occurrence of potential opportunistic bacteria on heavily polluted days.

Keywords Air quality · Airborne bacteria · Chile · Bioaerosols · Particulate matter · PM_{2.5}

Introduction

The air pollution as a result of an increase of particulate matter (dust, soot, cement, among others) and microbial components (bacteria, fungi, viruses and archaea) in the atmosphere (Cao et al. 2014), is a topic of concern around

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the globe. High fine particulate matter levels ($PM_{2.5}$, particles of 2.5 μm or less) can induce haze events with a negative impact on environmental and public health (Tomasi et al. 2017). In countries with air pollution issues (such as Chile), the $PM_{2.5}$ concentrations are constantly monitored and classified through a series of air quality indices (AQIs) to mitigate adverse effects on public health (MMA 2018). Therefore, there is great interest in characterizing the chemical constituents of the $PM_{2.5}$ fraction due to its consideration as the principal source of human health risks due to its small size, which can penetrate deeper into the respiratory system (Yang et al. 2020; Lai et al. 2019).

Bacteria are within the main components of airborne particles (Zhai et al. 2018), their relative abundance in urban areas on fine and ultrafine fractions range between 10^4 and 10^7 cells m^{-3} air (Wei et al. 2019b). Additionally, the diversity and abundance of total and pathogenic bacteria correlates positively with particles concentration and pollutants (Zhong et al. 2019; Li et al. 2019). Studies focused on air bacterial communities composition has shown that abundance and diversity are positively correlated with particulate matter concentrations, since particulate matter can provide niches, act as energy sources and carriers (Wei et al. 2019a; Dong et al. 2016; Smets et al. 2016; Zhai et al. 2018). Other studies have revealed strong correlations between airborne bacteria and meteorological factors, suggesting relative humidity and wind speed as main drivers of community structure (Xie et al. 2018; Zhong et al. 2019; Bai et al. 2020). Unstable compositions at vertical atmospheric height during a polluted sampling day have been reported (Bai et al. 2020); thus, the influence of environmental factors on the composition of airborne bacterial communities requires exploration under particular or specific meteorological conditions. In this sense, DNA fingerprint methods such as PCR-DGGE have proven to be particularly useful as a first approach for continuous, cheaper and rapid comparison analysis of airborne bacterial community analyses based on universal 16S rRNA genes (Tanaka et al. 2015; Qi et al. 2018; Maki et al. 2013).

Temuco is one of the most polluted cities in Chile and Latin America (IQAir AirVisual, 2018). Household emissions can reach to tenfold times the national average (Ministry of Environment 2018) during cold seasons (Jorquera et al. 2018). Moreover, residential firewood combustion represents 93% of pollutant emissions, associated with an increase in respiratory diseases during the winter (Saide et al. 2016). However, bacterial communities composition in the $PM_{2.5}$ of Temuco has still not been explored. This study analyzed and compared $PM_{2.5}$ bacteria present in different AQIs applied as environmental regulation by the Chilean Ministry of Environment (MMA) in accordance with World Health Organization (WHO) guidelines (WHO 2006). Additionally, the relationship between environmental factors

($PM_{2.5}$, PM_{10} , CO, temperature, wind speed, relative humidity and precipitation) and the bacterial community structure were also examined.

Materials and methods

Sampling

The $PM_{2.5}$ samples were collected in May 2018 from the Temuco environmental monitoring station “Las Encinas” (38°44'55.3"S 72°37'14.8"W), provided by regional MMA (Fig. S1). Samples were obtained using glass fiber filter low-volume air sampler (BAM 1020, Met One Instruments, Inc) equipped with $PM_{2.5}$ fractionating inlets. The five AQIs are: good (0–50 $\mu\text{g}/\text{m}^3$), moderate (51–79 $\mu\text{g}/\text{m}^3$), alert (80–109 $\mu\text{g}/\text{m}^3$), pre-emergency (110–169 $\mu\text{g}/\text{m}^3$) and emergency ($> 170 \mu\text{g}/\text{m}^3$). A total of fifteen samples were analyzed, from 3 different days and each AQI (3 days \times 5 AQIs = 15 samples) under sterile conditions in the Applied Microbial Ecology Laboratory (EMAlab) of Universidad de La Frontera. Local environmental conditions and factors: $PM_{2.5}$, PM_{10} , carbon monoxide (CO), temperature (T), wind speed (W), relative humidity (H) and precipitation (P) measurements were obtained from reports available for “Las Encinas” station in the MMA data platform (<http://airechile.mma.gob.cl/>).

DNA extraction and bacterial community composition

Samples were pretreated by vortexing for 1 h in 2 mL of 0.85% NaCl, then frozen in liquid nitrogen and grounded with mortar and pestle. Total DNA was extracted using PowerSoil® DNEasy Kit (QIAGEN, Inc.) according to the manufacturer’s instructions. Quality and quantity of DNA extracts were measured using a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific, Inc., USA). Samples absorbance ratios of ~ 1.8 were used for DGGE-PCR analysis of 16S rRNA genes, targeting V6-V8 regions with the universal primers set EUBf933-EUBr1387 (Iwamoto et al. 2000) coupled with a GC clamp as described by Jorquera et al. (2014). The PCR products (20 μL) were loaded into 6% (w/v) denaturing polyacrylamide gel of 45–65% gradient (urea: formamide). Electrophoresis was performed for 16 h at 60 °C and 100 V. The gel was stained in SYBR Gold (Invitrogen™, Thermo Fisher Scientific, Inc.) and photographed on an UV transilluminator. Representative bands were selected based on their intensity and dominance in DGGE gels to determine bacterial taxonomy, which were carefully excised, re-amplified and sent to sequencing by Macrogen, Inc. (Seoul, Korea). Sequences obtained were trimmed, cleaned and compared with GenBank database

using MEGABLAST (<http://blast.ncbi.nlm.nih.gov/>) (> 70% identity and 0.01 cutoff for E-values).

Data analysis

The relationship between particulate matter concentration and environmental factors was carried out by Spearman's correlation rank using SPSS software version 22 (IBM Inc., Armonk, NY, USA). Bacterial diversity was analyzed by Shannon diversity index (H') based on DGGE profiles according to Degens et al. (2000). Similarities between PCR-DGGE banding patterns and different pollution conditions were compared by dendrogram analysis as indicated by Acuña et al. (2013), based on distance matrices from CLIQS 1D Gel Image Analysis Software (TotalLab Ltd., Newcastle, UK). These matrices were also used for nonmetric multidimensional scaling (nMDS) analysis with PRIM-ERe v7 program (Primer-E Ltd., Ivybridge, UK). Statistical significance of the relationship among bacterial community structure and environmental factors was analyzed by distance-based redundancy analysis (dbRDA) using multivariate analysis for a linear model (DistLM). The environmental factors selected are summarized in Table 1.

Results and discussion

Bacteria are important components of the atmosphere that are dispersed and transported in air, together with particulate matter derived from anthropogenic and natural sources toward different habitats by aerosolization processes (Ruiz-Gil et al. 2020). Several studies have focused on decipher the compositions of airborne bacterial communities and their relationships with air pollution levels (Li et al. 2019; Pan et al. 2019; Zhang et al. 2016) and with the occurrence of respiratory diseases, such as asthma and allergies (Wu et al. 2018, 2019). To our knowledge, this study represents the first approach to characterizing the airborne bacterial communities present in particulate matter and their relationship with environmental factors under different air quality conditions in Temuco city during colder months.

Environmental characteristics during air sampling

Over the study period (May 2018), the daily mean $PM_{2.5}$ ranged from 28 to 241 $\mu\text{g}/\text{m}^3$ (Table 1). The PM_{10} and CO values increased to > 100 $\mu\text{g}/\text{m}^3$ and > 1.0 ppm during colder days compared with warmer days. The daily average T ($^{\circ}\text{C}$) and W (m s^{-1}) oscillated from 2.6 to 13.6 and from 0.40 to 3.26, respectively. The H (%) persisted over 90% during all sampling days, whereas P (mm) was observed on some days, ranging from 0.01 to 2.68 (Table 1).

Table 1 Environmental conditions in Temuco city during sampling days used in this study

| Sample code | Sampling date | Air quality | $PM_{2.5}^a$ ($\mu\text{g}/\text{m}^3$) | PM_{10}^a ($\mu\text{g}/\text{m}^3$) | Carbon monoxide ^a (ppm) | Temperature media ^b ($^{\circ}\text{C}$) | Wind speed ^b (m s^{-1}) | Relative humidity ^b (%) | Precipitation ^b (mm) |
|-------------|---------------|-------------|---|--|------------------------------------|---|---|------------------------------------|---------------------------------|
| GD_1 | 09-05-2018 | Good | 30 | 74 | 0.54 | 12.2 | 0.74 | 91 | 0 |
| GD_2 | 14-05-2018 | Good | 28 | 37 | 0.47 | 10.6 | 1.54 | 93 | 0.82 |
| GD_3 | 28-05-2018 | Good | 34 | 44 | 0.21 | 9.7 | 1.31 | 100 | 2.68 |
| MD_1 | 10-05-2018 | Moderate | 51 | 46 | 0.75 | 12.0 | 0.60 | 93 | 0 |
| MD_2 | 13-05-2018 | Moderate | 57 | 74 | 0.28 | 13.2 | 3.26 | 91 | 0.48 |
| MD_3 | 27-05-2018 | Moderate | 57 | 68 | 0.57 | 9.8 | 2.64 | 94 | 1.31 |
| AL_1 | 12-05-2018 | Alert | 93 | 117 | 1.07 | 9.9 | 0.88 | 98 | 0 |
| AL_2 | 16-05-2018 | Alert | 90 | 106 | 0.49 | 7.8 | 0.87 | 98 | 1.06 |
| AL_2 | 19-05-2018 | Alert | 90 | 107 | 0.64 | 7.1 | 0.63 | 90 | 0.01 |
| PE_1 | 21-05-2018 | PreEme | 155 | 166 | 1.85 | 4.8 | 1.16 | 92 | 0.02 |
| PE_2 | 25-05-2018 | PreEme | 121 | 145 | 0.67 | 7.5 | 1.21 | 90 | 0.02 |
| PE_3 | 31-05-2018 | PreEme | 116 | 125 | 1.54 | 4.0 | 1.00 | 90 | 0.02 |
| EM_1 | 22-05-2018 | Emergency | 183 | 204 | 2.48 | 2.6 | 0.61 | 100 | 0 |
| EM_2 | 23-05-2018 | Emergency | 241 | 273 | 2.53 | 3.9 | 0.40 | 95 | 0 |
| EM_3 | 24-05-2018 | Emergency | 182 | 217 | 1.44 | 6.3 | 0.68 | 95 | 0 |

^a $PM_{2.5}$ (particulate matter with an aerodynamic diameter of 2.5 μm or less), PM_{10} (particulate matter with an aerodynamic diameter of 10 μm or less), and carbon monoxide data were taken from <http://airechile.mma.gob.cl>

^bTemperature, wind speed, relative humidity and precipitation data were taken from <https://climatologia.meteochile.gob.cl>

Table 2 Spearman correlation coefficients between particulate matter and environmental conditions in Temuco city during sampling days used in this study

| | PM _{2.5} | PM ₁₀ | CO | Temperature | Wind speed | Relative humidity | Precipitation |
|-------------------|-------------------|------------------|---------|-------------|------------|-------------------|---------------|
| PM _{2.5} | 1 | 0.966** | 0.846** | - 0.843** | - 0.444 | 0.288 | - 0.373 |
| PM ₁₀ | | 1 | 0.835** | - 0.788** | 0.492 | - 0.196 | - 0.483 |
| CO | | | 1 | - 0.754** | - 0.618* | 0.162 | - 0.552* |
| Temperature | | | | 1 | 0.432 | - 0.225 | 0.063 |
| Wind speed | | | | | 1 | - 0.286 | - 0.694** |
| Relative Humidity | | | | | | 1 | - 0.128 |
| Precipitation | | | | | | | 1 |

PM_{2.5} Particulate matter with an aerodynamic diameter of 2.5 µm or less, PM₁₀ Particulate matter with an aerodynamic diameter of 10 µm or less, CO Carbon monoxide

*Denotes a correlation significant at the 0.05 level

**Denotes a correlation significant at the 0.01 level. 0.01

Correlation analysis showed that PM_{2.5} concentration has a strong positive correlation with PM₁₀ and CO concentrations ($R=0.966$, 0.846), whereas a strong negative correlation was observed with T ($R=-0.843$) (Table 2). Moreover, PM₁₀ and CO also exhibited strong positive correlations between them ($R=0.835$) and strong negative with T ($R=-0.788$, -0.754). Fu et al. (2020) showed that PM_{2.5} is positively correlated with CO during polluted days in China. It is also commonly observed that the spatial distribution of CO and particulate matter concentration tends to be the same in most parts of the day (Wang et al. 2017; Chuai and Feng 2019). However, it has been suggested that the correlation between PM_{2.5} and meteorological factors varies according to temporal and seasonal scales (Yang et al. 2017). In Temuco city, the emissions of PM_{2.5} are based on residential

firewood combustion for heating purposes; therefore, a combination of more sustainable heating solution developments and politics is required to regulate and mitigate the effects of PM_{2.5} release (Jorquera et al. 2018).

Bacterial community composition

The DGGE banding profiles of the bacterial communities differed among samples and AQIs (Fig. 1a). The majority of representative 16S rRNA gene sequences obtained from the excised DGGE bands showed high similarities to members of the Proteobacteria phylum (Table 3). Under emergency conditions, a higher number of bands were found in comparison with other samples and the majority of bands presented high homology with members of Sphingomonadaceae,

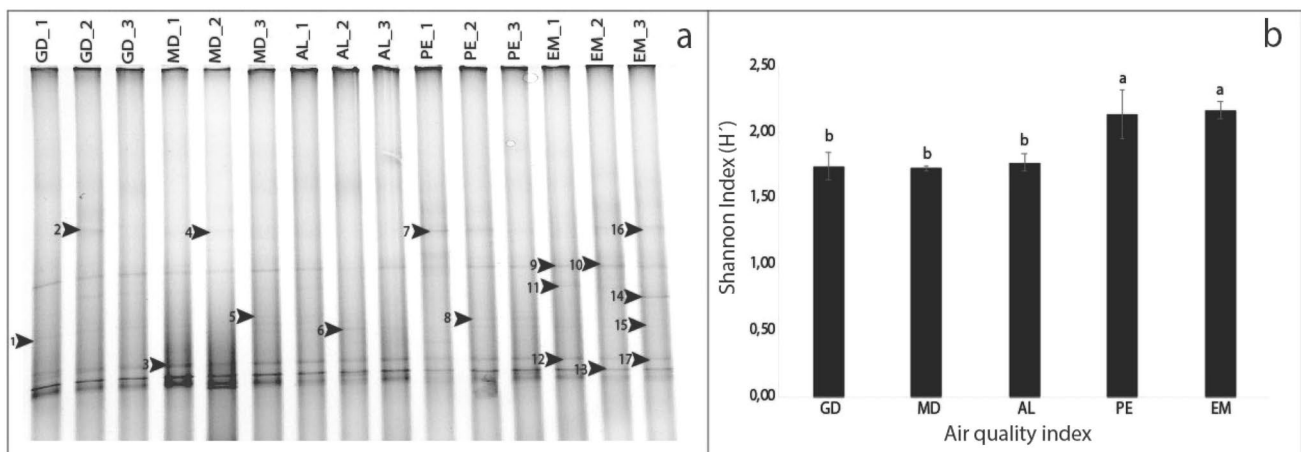


Fig. 1 a Denaturing gradient gel electrophoresis (DGGE) banding profiles of bacterial communities associated with PM_{2.5} during diverse air quality conditions in Temuco city. Arrowheads and numbers indicate representative bands that were excised for DNA sequencing analysis. b Shannon diversity indices (H') of bacterial communities generated by the PRIMERe v7 program ([http://www.](http://www.primer-e.com/)

[primer-e.com/](http://www.primer-e.com/)). Air quality index Good: GD_1, GD_2, and GD_3; Moderate: MD_1, MD_2, and MD_3; Alarm: AL_1, AL_2, and AL_3; Pre-emergency: PE_1, PE_2, and PE_3; Emergency: EM_1, EM_2, and EM_3. The bars represent standard error, and the same letter denotes no significant difference ($p \leq 0.05$, Tukey's multiple range test)

Alteromonadaceae and Rhodobacteraceae families (Table 3). Bands from pre-emergency and alert AQIs samples presented high similarity with Enterobacteriaceae, Alteromonadaceae and Sphingomonadaceae families (88 and 92% identity). In addition, obtained bands from samples collected during moderate and good air conditions, were characterized as members of Pseudomonadaceae and Phyllobacteriaceae families (81 ~ 95%). Shannon diversity index revealed significant differences ($P < 0.05$) between pre-emergency and emergency levels in comparison with good, moderate and alert conditions (Fig. 1b).

In this study, dendrogram analysis revealed the presence of two clusters: one of them composed of samples collected under good, moderate and alert conditions, and the other during emergency and pre-emergency conditions at a 75% similarity cutoff (Fig. 2a). At 85% similarity, the alert samples were sub grouped. Further nMDS analysis (Fig. 2b) revealed a greater separation between bacterial communities in samples obtained under good and moderate air conditions (80% similarity) and samples collected under emergency and environmental pre-emergency conditions. Lee et al. (2009) reported variations in DGGE band

Table 3 Phylogenetic assignment of representative bands excised from denaturing gradient gel electrophoresis (DGGE)

| Origin/band ^a | Taxonomic group ^b | Closest relatives or cloned sequences (accession no.) ^c | Similarity (%) | Accession no. |
|----------------------------|---|--|----------------|---------------|
| Good (band no. 1) | Unclassified bacteria | Uncultured bacterium sp. from snow around Russian Antarctic stations (JX855459) | 95.1 | MW364621 |
| Good (band no. 2) | Proteobacteria | Uncultured delta proteobacterium from Ferri-rich Mats and Basaltic Rock (FJ497423) | 82.1 | MW364620 |
| Moderate (band no. 3) | Proteobacteria; Alphaproteobacteria; Rhizobiales; Phyllobacteriaceae | <i>Mesorhizobium</i> sp. from phosphate sand (JF745869) | 81.2 | MW364619 |
| Moderate (band no. 4) | Unclassified bacteria | Uncultured bacterium sp. from subsurface aquifer sediment (JX855447) | 98.3 | MW364618 |
| Moderate (band no. 5) | Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae | <i>Pseudomonas putida</i> sp. from farm soil (JX844646) | 84.2 | MW364617 |
| Alert (band no. 6) | Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae | <i>Novosphingobium</i> sp. from river water (KJ685948) | 95.3 | MW364616 |
| Pre-emergency (band no. 7) | Proteobacteria; Gammaproteobacteria; Alteromonadales; Alteromonadaceae | <i>Alteromonas</i> sp. associated with <i>Montastrea annularis</i> (FJ952818) | 92.1 | MW364615 |
| Pre-emergency (band no. 8) | Proteobacteria; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae | <i>Enterobacter ludwigii</i> sp. associated with <i>Hieracium piloselloides</i> (MF765332) | 88.5 | MW364614 |
| Emergency (band no. 9) | Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae | <i>Novosphingobium</i> sp. from river water (KJ685948) | 95.8 | MW364613 |
| Emergency (band no. 10) | Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae | <i>Novosphingobium</i> sp. from river water (KJ685948) | 98.7 | MW364612 |
| Emergency (band no. 11) | Proteobacteria; Alphaproteobacteria | Uncultured alpha proteobacterium from natural Asphalt Lake (GU120589) | 98.7 | MW364611 |
| Emergency (band no. 12) | Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae | <i>Novosphingobium</i> sp. from water surrounding roots of a beech tree | 95.6 | MW364610 |
| Emergency (band no. 13) | Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae | <i>Sphingomonas</i> sp. from printing paper machines (AJ001052) | 86.2 | MW364609 |
| Emergency (band no. 14) | Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae | Uncultured <i>Novosphingobium</i> sp. from KSC-Astrotek' spacecraft during DAWN mission (FJ193864) | 96.7 | MW364608 |
| Emergency (band no. 15) | Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae | Uncultured <i>Rhodobacteraceae</i> sp. from diatom-bacteria-virus model system (KM383313) | 87.3 | MW364607 |
| Emergency (band no. 16) | Proteobacteria; Gammaproteobacteria; Alteromonadales; Alteromonadaceae; | <i>Alteromonas</i> sp. from seagrass beds and surrounding areas in the Seto Inland Sea, Japan (LC186466) | 87.3 | MW364606 |
| Emergency (band no. 17) | Proteobacteria; Alphaproteobacteria; Sphingomonadales; Sphingomonadaceae | <i>Novosphingobium</i> sp. from river water (KJ685948) | 84.7 | MW364605 |

^aCorresponding DGGE bands shown in Fig. 2a

^bThe phylogenetic assignment is based on non-redundant GenBank database from NCBI (<http://www.ncbi.nlm.nih.gov>)

^cBased on partial sequencing of 16S rRNA gene and comparison with those present in GenBank using Blastn and Megablast

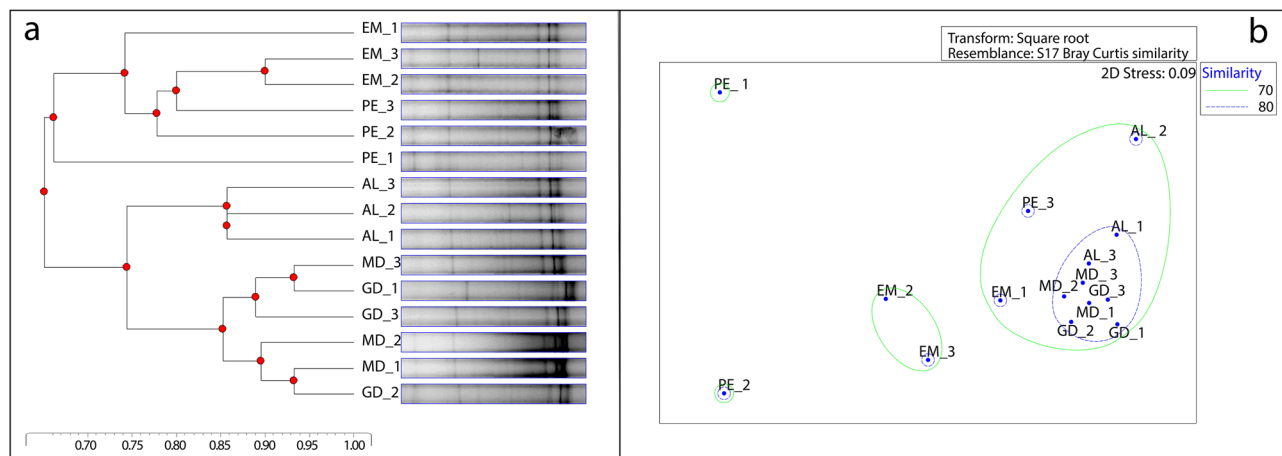


Fig. 2 **a** Dendrogram of DGGE profiles generated by Phoretix 1D Pro Gel Analysis Software (<http://totalab.com/>). **b** Nonmetric multidimensional scaling (NMDS) analysis of DGGE profiles generated by the PRIMERe v7 program (<http://www.primer-e.com/>). Air qual-

ity index Good: GD_1, GD_2, and GD_3; Moderate: MD_1, MD_2, and MD_3; Alarm: AL_1, AL_2, and AL_3; Pre-emergency: PE_1, PE_2, and PE_3; Emergency: EM_1, EM_2, and EM_3

patterns with 23% similarity in 30 atmospheric samples from dust events in Korea. It has been described that the similarity of bacterial communities can increase in polluted urban areas where $PM_{2.5}$ concentrations are high (Liu et al. 2019; Jeon et al. 2011). In general, nMDS analysis showed a high similarity (80%) between all the analyzed samples (Fig. 2b); however, similarity among samples and season was reported to be low (approximately 10–40%) in Toyama city (Japan) by Tanaka et al. (2015) and in Milan city (Italy) by Bertolini et al. (2013). Negrin et al. (2007) found a higher similarity of the airborne bacterial community in winter (50–87%) than in spring and autumn (32–80% and 19–92%, respectively) in Argentina. Thus, airborne bacterial community studies should be carried out using temporal and seasonal variability scales.

The airborne bacterial community was dominated by members of the Proteobacteria phylum, with Sphingomonadales and Enterobacterales being the most abundant orders (Table 3). Members of Proteobacteria have been described as typical airborne populations present at several latitudes (Tanaka et al. 2019; Wang et al. 2018; Koch et al. 2019; Li et al. 2019). The occurrence of the Sphingomonadales order has been associated with antibiotic-resistance genes by Wang et al. (2019), where tetracycline-resistance genes were dominant in the atmosphere and presumably harbored by *Sphingomonas*. In addition, these authors also revealed that the number of antibiotic-resistant genes increased with suitable conditions for bacterial proliferation, such as high humidity and smog levels (Wang et al. 2019). As aerosols can penetrate the pulmonary system and are highly transmissible in the atmosphere, airborne resistance genes represent a risk for livestock and public health (Ruiz-Gil et al. 2020).

Notably, the *Novosphingobium* genus was identified at all air quality levels, except pre-emergency, showing a percentage of identity between 91 and 100%. Classified as an opportunistic genus, *Novosphingobium* has been associated with chronic obstructive pulmonary diseases (Rutebemberwa et al. 2014). Moreover, the *Novosphingobium* and *Alteromonas* genera are able to catabolize dissimilar organic compounds (phenol, aniline, and polysaccharides) abundant in airborne particles (Wang et al. 2018; Koch et al. 2019). Wei et al. (2019a) found that higher concentrations of gas pollutants can provide considerable amounts of nutrients, enabling a high abundance of organic pollutant-degrading bacteria. Considering the aforementioned factors, the occurrence and abundance of potential opportunistic bacteria associated with fine particulate matter should be the priority in atmospheric microbiome studies (Table 3).

Relationship between environmental factors and airborne bacterial communities

According to dbRDA analysis, the airborne bacterial community structures present in all samples were clustered into two groups according to the AQI (Fig. 3). The examined environmental factors could explain 56.1% of the total variations in the airborne bacterial community structure (data not shown). dbRDA 1 and dbRDA 2 explained 33.2% and 9.3% of the airborne bacterial community variations, respectively. The airborne bacterial structure was strongly correlated with $PM_{2.5}$ concentrations ($P < 0.01$), and this factor could explain 33.2% of the total variation.

The $PM_{2.5}$ concentrations was the most significant factor associated with airborne bacterial community structure

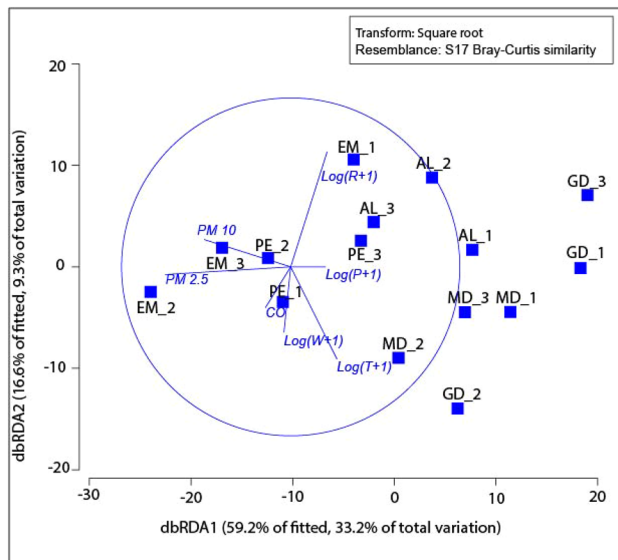


Fig. 3 Distance-based redundancy analysis (dbrDA) of variation in airborne bacterial community profiles as explained by environmental parameters generated by the PRIMERe v7 program (<http://www.primer-e.com/>). Vectors represent correlations of variables with community structures along the first two dbrDA axes. Dots represent bacterial community profiles present in PM_{2.5} during diverse air quality conditions. Arrows represent environmental parameters PM_{2.5}, PM₁₀, CO: carbon monoxide, T: temperature, W: wind speed, RH: relative humidity, P: precipitation. The values in parentheses indicate the percentages of the fitted and total variations explained by each axis. Air quality index Good: GD₁, GD₂ and GD₃; Moderate: MD₁, MD₂ and MD₃; Alarm: AL₁, AL₂ and AL₃; Pre-emergency: PE₁, PE₂, and PE₃; Emergency: EM₁, EM₂, and EM₃

(Fig. 3) as has been reported in other studies (Alghamdi et al. 2014; Dong et al. 2016). Moreover, airborne culturable bacteria community was also found to be higher and positively correlated with particulate matter concentration and chemical composition in Asian dust events (Jeon et al. 2011). Chemical components of aerosols, such as CO and CO₂, have shown a higher positive correlation with the bacterial community, explaining approximately 55% of the variance (Innocente et al. 2017) associated with firewood combustion (Fu et al. 2020; Xu et al. 2017; Zhong et al. 2019; Li et al. 2019). Our findings show that despite the high pollution and carbon concentration in particulate matter produced in Temuco during colder days and due to residential firewood combustion, the bacterial community can be detected, and its diversity increases with air quality deterioration. This research is projected in the future to extend to outdoor and indoor (built environments) studies of other microbial entities suspended in the air, such as public health-relevant fungi (*Aspergillus*) and viruses (SARS-CoV-2), using high-throughput sequencing platforms. In addition, culturing the bacteria present in the airborne particulate matter would reveal those bacterial cells that remain viable in

the atmosphere as well as their metabolic activity, which could have a more accurate positive or negative correlation with respiratory diseases associated with colder months than identification by total DNA extraction only.

Conclusions

The use of the denaturing gradient gel electrophoresis (DGGE) technique allowed the first approach to determine the airborne bacterial community structures present in fine particulate matter (PM_{2.5}) under different air quality conditions in Temuco city during autumn days, with greater diversity during environmental emergencies. Positive correlations were observed between PM_{2.5} and PM₁₀ as well as CO, suggesting that PM_{2.5} responds to other air pollutants. Most of the sequenced representative DGGE bands were classified as members of the Proteobacteria phylum, and *Novosphingobium* was the most dominant genus present in the airborne bacterial community. In addition, airborne bacterial community composition correlated with PM_{2.5} and other air pollutants, suggesting that atmospheric factors can modulate airborne microbes in the Temuco urban area. Future research should address the composition and activity of airborne bacterial communities and their environmental influencing factors as well as their impact on public health and ecosystem functions. Future research could also be relevant to the revision or redesign of Chilean environmental regulations on air quality at the local and national levels.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00203-021-02740-6>.

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Authors contributions TRG, LM, EC, and JJA designed the study and contributed to the data collection. JR and MC analyzed and interpreted the data. FCA and JJA carried out the statistical analyses and interpretation of the results. JJA, SF and MAJ writing-review and editing the manuscript. All authors approved the submitted manuscript.

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