



Global dispersal and potential sources of antibiotic resistance genes in atmospheric remote depositions

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ABSTRACT

Antibiotic resistance has become a major Global Health concern and a better understanding on the global spread mechanisms of antibiotic resistant bacteria (ARB) and intercontinental ARB exchange is needed. We measured atmospheric depositions of antibiotic resistance genes (ARGs) by quantitative (q)PCR in rain/snow collected fortnightly along 4 y. at a remote high mountain LTER (Long-Term Ecological Research) site located above the atmospheric boundary layer (free troposphere). Bacterial composition was characterized by 16S rRNA gene sequencing, and air mass provenances were determined by modelled back trajectories and rain/snow chemical composition. We hypothesize that the free troposphere may act as permanent reservoir and vector for ARB and ARGs global dispersal. We aimed to *i*) determine whether ARGs are long-range intercontinental and persistently dispersed through aerosols, *ii*) assess ARGs long-term atmospheric deposition dynamics in a remote high mountain area, and *iii*) unveil potential diffuse ARGs pollution sources. We showed that the ARGs *sulI* (resistance to sulfonamides), *tetO* (resistance to tetracyclines), and *intI1* (a proxy for horizontal gene transfer and anthropogenic pollution) were long-range and persistently dispersed in free troposphere aerosols. Major depositions of tetracyclines resistance matched with intensification of African dust outbreaks. Potential ARB mostly traced their origin back into agricultural soils. Our study unveils that air masses pathways are shaping ARGs intercontinental dispersal and global spread of antibiotic resistances, with potential predictability for interannual variability and remote deposition rates. Because climate regulates aerosolization and long-range air masses movement patterns, we call for a more careful evaluation of the connections between land use, climate change and ARB long-range intercontinental dispersal.

1. Introduction

Antibiotic resistance (AR) is a major public health concern at both local and global scales (Hernando-Amado et al. 2019). The dissemination of AR among bacterial pathogens, exemplified by the rapid dispersal of carbapenem-resistant Enterobacteriaceae (CREs) across countries and continents (McKenna 2013), undermines the efficacy of many antibiotic therapies, switching a common infection into a life-threatening major concern. Although AR is an ancient phenomenon (Allen et al. 2010; D'Costa et al., 2011; Davies and Davies 2010), the overuse and misuse of antibiotics in clinical, veterinary, and animal husbandry practices is considered the major cause for the widespread increase of AR among microorganisms (Goossens et al. 2005; Zhu et al. 2013). Anthropogenic

activities related to the inaccurate management of waste (sewage, sludge, antibiotic residues) and unsuitable practices in crop fertilization create major environmental reservoirs of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) (Allen et al. 2010; Davies and Davies 2010; Vaz-Moreira et al. 2014). Recent studies have revealed direct associations between the prevalence of resistances in common pathogens and the increase in both local temperatures and human population density (Kaba et al. 2020; MacFadden et al. 2018). Anthropological and socioeconomic factors (e.g. poor sanitation and corrupt governance) have also been identified as important contributors to the prevalence of AR, especially in low-income countries (Collignon et al. 2018).

Several studies suggest that ARGs can be exchanged via horizontal

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gene transfer among bacteria associated to livestock and soil, and between bacterial pathogens of animals and humans, thus intimately linking the environmental and the clinical resistome (Forsberg et al. 2012; Smillie et al. 2011). Processes that exacerbate the spread and interactions between these biological compartments may therefore promote AR dispersal among the total environment, including atmosphere, hydrosphere, biosphere, lithosphere, and the anthroposphere, and are of key relevance for studies under the One Health approach (Hernando-Amado et al. 2019). ARB and associated ARGs can be aerosolized and have consistently been detected in local air samples collected from a wide repertoire of environments such as wastewater treatment plants (Li et al. 2016; Teixeira et al. 2016), composting plants (Gao et al. 2018), livestock farming (Chapin et al. 2005; Gibbs et al. 2006), and in industrial, urban and rural areas (Li et al. 2018; Xie et al. 2018; Zhang et al. 2019; Zhou et al. 2018). In fact, soils are considered one of the largest reservoirs of AR, and changes in land use have multiple consequences for environmental and human health (D'Costa et al. 2006; Forsberg et al. 2014). ARGs were also detected in dust storms in one study in the Eastern Mediterranean (Mazar et al. 2016).

It has been well reported that strong air currents mobilize microscopic particles up to the high atmosphere, which can be transported thousands kilometers away from the original source (Després et al. 2012; Kellogg and Griffin 2006). Aerosolized microorganisms attached to dust particles are able to travel between continents and survive the harsh conditions prevailing in the middle-to-upper atmosphere (Hervás et al., 2009; Smith et al. 2013). Physical forces, such as air masses circulation and oceanic currents, globally disperse toxics and microorganisms and connect distant and remote environments thus threatening the Global Health (Hernando-Amado et al. 2019). However, whether or not the free troposphere can be a successful conduit for the long-range dispersal of resistances leading to intercontinental and inter-ecosystems remote connections, remains largely unexplored. In the present investigation, we studied washout aerosols present in both rain and snow samples collected during 4-years in a remote high-altitude mountain of the Central Pyrenees (Long-Term Ecological Research site AigüesTortes, NE Spain) to study the potential for long-range dispersal and temporal recurrence of airborne ARGs. The LTER-AT site was placed above the atmospheric boundary layer (free troposphere) to discriminate between local and long-range atmospheric deposition. We applied real time quantitative PCR (qPCR) to quantify six well-known ARGs (*bla*_{TEM}, *bla*_{CTX-M}, *tetM*, *tetO*, *sulI* and *ermB*) and the gene *intI1* encoding the integrase of Class I integrons involved in gene capture and transfer. The airborne bacteria were also characterized using high throughput amplicon-targeted sequencing of the 16S rRNA gene. Air masses and atmospheric precipitations were characterized by modelled back trajectories and supported by previously reported chemical composition analyses (Cáliz et al. 2018). Hence, our dataset was unique to explore together for the first time long-range persistence, temporal occurrence, global dispersal mechanisms, and remote deposition of airborne ARGs present in rain and snow.

2. Methods

2.1. Study site and sampling

Atmospheric precipitation (rain and snow) was collected with an automatic MTX ARS 1010 (MTX Italia SPA, Modane, Italy) passive sampler equipped with a hygroscopic sensor cell. The wet container (a 667 cm² area polyethylene bucket) was protected with a metallic articulated lid that activated only under rain/snow conditions. The sampler was placed at c. 1,700 MASL within the protected Aigüestortes i Estany de Sant Maurici National Park (42°33'N 0°53'E, Central Pyrenees, NE Spain), a placement above the atmospheric boundary layer prevailing in this area (c.1500 MASL) (Ontiveros et al. 2021). The wet container remained fully covered and in the dark preventing atmospheric inputs until the hygroscopic sensor was activated. Water samples from the wet

container were filtered using pre-combusted (450 °C, 4 h) Whatman GF/F filters and then dried in a laboratory oven for 4 h at 40 °C, and aseptically kept in a dark and dry place. The containers were carefully cleaned, dried and repeatedly washed with sterile MilliQ water after each use and internal controls ruled out potential cross-contaminations. We had previously tested that wet depositions collected above the boundary layer can substantially reduce near-surface aerosols contamination, and can be a cost-effective useful proxy for monitoring inter-continental exchange of microbes from the high-atmosphere (Triadó-Margarit et al. 2019, Ontiveros et al. 2021), despite the picture provided on the tropospheric microbiota can be incomplete (Els et al. 2019). An automatic weather station was located next to the collector (meteorological information available at <http://loopweb.org/loopweb2018/index.php/lter-research/meteorological-data>). Time-integrated samples were mostly collected every 15 days, from June 2010 to October 2013. For convenience, the dataset was divided in two seasons: "winter" (from November to March, n = 25) and "summer" (from May to September, n = 39).

2.2. Quantification of antibiotic resistance genes

DNA was extracted using the Mobio PowerSoil DNA Isolation Kit (Mobio Laboratories) according to manufacturer's instructions, and aliquots were stored separately at -80 °C. DNA concentrations were measured using Qubit 2.0 fluorometer (Life Technologies; Carlsbad, CA, USA). Abundances of six genes encoding resistance to β -lactams (*bla*_{TEM}, *bla*_{CTX-M}), tetracyclines (*tetM*, *tetO*), sulfonamides (*sulI*) and macrolides (*ermB*), and the 16S rRNA gene (as a proxy of bacterial abundance) were determined using qPCR as previously described (Subirats et al. 2017). These ARGs were selected to cover both different resistance mechanisms for clinically relevant antibiotics and bacterial hosts widely distributed. Besides, the Class I integron-integrase gene (*intI1*) was quantified as a proxy for horizontal gene transfer and anthropogenic pollution (Gillings et al., 2015). Ten-fold dilutions of linearized plasmid DNA containing known concentration of target genes were used for the standard curves, which were generated by cloning into *E. coli* the amplicon from positive controls using pCR2.1-TOPO vector system (Invitrogen, Carlsbad, CA, USA) following manufacturer's instructions (Invitrogen). Number of gene copies were calculated for each standard curve as previously described (Subirats et al. 2017) and three replicates for each dilution were measured. All qPCR assays were carried out in duplicate using SYBR green detection chemistry on a MX3005 system (Agilent Technologies; Santa Clara, CA, USA). Each reaction contained 12.5 μ L of either 2x Brilliant III UltraFast QPCR Master Mix (Stratagene, La Jolla, CA, USA) for *bla*_{TEM} or SYBR®Green Master Mix (Applied Biosystems) for the rest of the genes analyzed, 200 nM each forward and reverse primer and 10 ng of the DNA template. The final volume was adjusted to 30 μ L with DNase-RNase-free water. Details on cycling conditions and primer sets used are shown in Supplementary Table S1. Ten-fold serial dilutions of the standards were run in parallel along the samples and negative controls. Specificity of amplification was determined by analysis of the melting curves and gel electrophoresis of amplified products. All qPCR assays were carried out with high R² values (average 0.998), high efficiencies (91–97%) and a dynamic range of at least 5 orders of magnitude.

2.3. Calculation of potential gene deposition rates

Potential deposition rates were calculated as copies of genes per m² and per day, as recently described (Reche et al. 2018). Total gene counts normalized by the analyzed sample volume, the volume of rain/snow in the wet collector, the area (A) of the collector, and the length of the deposition period were considered for further scaling-up calculations as follows:

$$\text{Deposition rate} = \frac{\text{gene counts} / \text{mL-collector vol(mL)}}{A(\text{m}^2) \cdot \text{time (day)}}$$

These rates do not consider the additional effect of dry deposition. Dry depositions had very low microbial biomass and most of the samples were below detection limits (data not shown).

2.4. Air microbiota and 16S rRNA gene identification of putative bacteria carrying ARGs

High-throughput sequencing of 16S rRNA gene amplicons was carried out according to the genomic core facilities and methods of the RTSF-MSU (Michigan State University, USA) (<https://rtsf.natsci.msu.edu/>) using the Illumina MiSeq platform with 2x250 chemistry. The V4 region (c.a. 250 nucleotides) was amplified using primers F515-R806 (Caporaso et al. 2010). Raw sequences were clustered into operational taxonomic units (OTUs) at the 97% identity cutoff using the UPARSE pipeline (Edgar 2013) as previously described (Cáliz et al. 2018). Taxonomic assignment was carried out with the RDP classifier at a confidence threshold of 0.5 (Wang et al. 2007) against the SILVA reference database (release 119) (Quast et al. 2013). Sequences classified as mitochondria or chloroplast were excluded from downstream analysis. In order to minimize effects of sampling effort, samples were normalized to a depth of 12,500 reads using the average of multiple rarefactions. Raw sequences are available at the European Nucleotide Archive within the study project PRJEB14358.

Identification of airborne bacterial taxa potentially carrying ARGs was carried out after correlation analysis between 16S rRNA OTUs and specific ARGs abundances. OTUs deposition was normalized with the deposition of 16S rRNA gene copies per m² and per day. OTUs occurring in < 5 samples and samples with ARGs below detection limits were not included in the correlational analysis. The environmental sources was approached by Environmental Ontology (ENVO) terms after BLASTn closest match analyses against GenBank using the SEQenv pipeline (Sinclair et al. 2016) as previously described (Cáliz et al. 2018).

2.5. Data processing and statistical analyses

The origin of each precipitation event was determined using the Vertical Velocity NOAA HYSPLIT Model and GDAS meteorological data as previously reported (Cáliz et al. 2018). Backward trajectories were grouped per season using the clustering module implemented in HYSPLIT 4.0. The grouped trajectories showed the main directions and wind speeds at 3,000 MASL on the LTER-AT site. Estimated environmental origins were in agreement with chemical analyses of the rain and snow samples previously reported (Cáliz et al. 2018) where a Factor Analysis (FA) using Varimax rotation had reduced the chemical dataset to three previously identified main factors: factor one (NO₃⁻, SO₄²⁻, NH₄⁺, DOC) indicative of pollution; factor two (pH, ANC, DIC, Ca²⁺) presence of terrestrial aerosols, and factor three (Cl⁻ and Na⁺) indicative of marine origin (Izquierdo et al. 2012) (Supplementary Fig. S1). Saharan dust intrusion events for Northeast Spain were obtained from www.calima.ws (currently at <https://ess.bsc.es/bsc-dust-daily-forecast>).

All statistical analyses were run in the R environment (<http://www.r-project.org/>). Community ecology parameters were calculated using the package *vegan* (Oksanen et al., 2013) and graphs plotted using the package *ggplot2* (Wickham 2009). For distance-based community analyses, we calculated Bray-Curtis dissimilarities after Hellinger standardization (Legendre and Gallagher 2001). The multivariate homogeneity of group dispersions (variances in beta-diversity) and permutational multivariate analysis of variance (PERMANOVA) were used to test for seasonality on community similarity patterns. The Quantitative Insights Into Microbial Ecology toolkit –QIIME – (Caporaso et al. 2010) was also used to generate rarefied alpha-diversity metrics, including OTU richness (unique OTUs) and diversity (Shannon Index and Faith's phylogenetic diversity–PD). Hypothesis contrast tests and

correlations were carried out in R using the package *stats*; non-parametric tests were used when the assumptions for the parametric equivalents were not achieved. Student's *t*-test and non-parametric Mann-Whitney *U* test (or Kruskal-Wallis test) were used to determine differences between two (or more than two) sample groups. Pearson's Chi-squared test with Yates' continuity correction was used for qualitative variables. Spearman (*r*_s) coefficients were calculated to estimate the degree of correlation between the ARG abundances and quantitative variables. The false discovery rate (FDR) was applied to adjust probability (*p*) values for multiple comparisons of ARG and specific OTUs.

3. Results

3.1. Atmospheric transport patterns and microbiota composition

Cluster analysis showed the main wind trajectories reaching the Pyrenees in summer (seven, Fig. 1, panel A) and winter (eight, Fig. 1, panel B), respectively. Predominant atmospheric transport pathways were North and North-Eastern (Europe), North-Western and Western (Atlantic), South-Eastern and South-Western (Africa), and Regional recirculation (Fig. 1C). The frequency of trajectories on the LTER-AT site showed that air masses from the Atlantic predominated in winter (37%), while flows with regional recirculation prevailed in summer (39%). Although less frequently, depositions from continental Europe were higher during winter. Conversely, atmospheric depositions coming from Africa were similar in both seasons.

We identified a total of 2,241 airborne bacterial OTUs collected across all samples. Bacterial microbiota showed a high inter-similarity for the summer samples (variances in beta-diversity; *p* < 0.001, PERMANOVA; *R* = 0.17 and *p* < 0.001, Supplementary Fig. S2) whereas winter samples were richer and more diverse (*t*-test; *p* < 0.05 for OTU richness and Shannon, and *p* < 0.001 for Faith's PD, Supplementary Fig. S3). Prevalent OTUs in the air microbiota mostly affiliated to classes Alpha- and Betaproteobacteria followed by Gammaproteobacteria (Supplementary Fig. S4).

3.2. Dynamics of ARGs and potential ARB

Presence of genes *sul1*, *tetO* and *intI1* were observed in up to 60% of the samples analyzed (Supplementary Table S2), being *intI1* the most frequently detected gene. In turn, genes *bla*_{TEM}, *bla*_{CTX-M}, *tetM*, and *ermB* were always below detection limits. Genes *intI1* and *tetO* were evenly detected in both seasons, whereas *sul1* showed higher frequency in summer (*p* < 0.01, Chi-squared test). Concentrations of ARGs normalized according to the abundance of total bacteria, were higher in winter than in summer (Supplementary Fig. S5).

Surface potential deposition rates (gene copies per m² per day) of 16S rRNA genes related to wet precipitation ranged between 2.60 × 10⁵ and 2.44 × 10¹⁰, with consistently higher abundances found in summer (*p* < 0.01, Mann-Whitney *U* test; Fig. 2A). Both ARGs and 16S RNA genes depositions were positively related, with the strongest correlation observed for *intI1* (*p* < 0.001, *r*_s = 0.80). Potential deposition rates averaged 1.02 × 10⁶ gene copies per m² per day for *intI1*, 8.74 × 10⁴ for *sul1*, and 1.04 × 10⁵ for *tetO*. Depositions of both *intI1* and *sul1* were higher in summer (*p* < 0.05 and *p* < 0.001, Mann-Whitney *U* test, respectively; Fig. 2B and C). Conversely, *tetO* did not show seasonal trends (Fig. 2D). We also observed that *sul1* and *tetO* showed higher variation for the interannual deposition rates (*p* < 0.05, Kruskal-Wallis test). The deposition rates of *intI1* and 16S rRNA genes did not have consistent interannual differences but showed a significant signal with rain/snow chemical composition and correlated significantly with main factor one (pollution) (*p* < 0.05, *r*_s = 0.32 and *p* < 0.01, *r*_s = 0.33, respectively) and two (terrestrial origin) (*p* < 0.01, *r*_s = 0.42 and *p* < 0.05, *r*_s = 0.26, respectively). Deposition rates of *sul1* showed positive correlations with pH (Fig. 3A), and *tetO* with rain/snow content of calcium and sulfate (Fig. 4A).

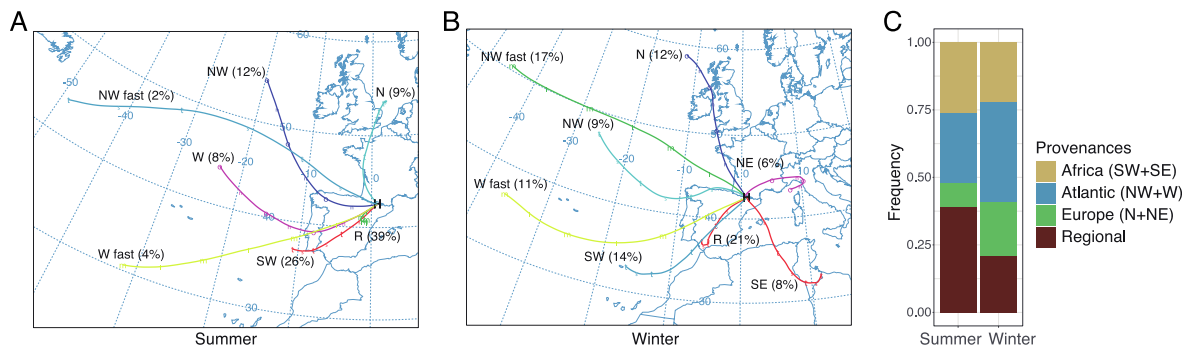


Fig. 1. Cluster centroids and relative frequency of associated back-trajectories for all precipitation events from June 2010 to September 2013 in (A) summer and (B) winter. (C) Frequency of clusters associated to main provenances for both seasons.

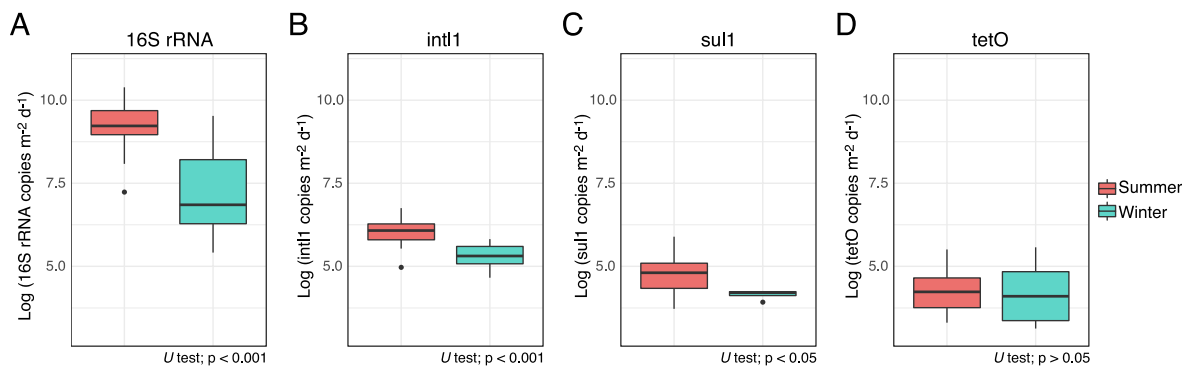


Fig. 2. Comparison of the deposition rates between summer and winter periods of (A) 16S rRNA, (B) *int1*, (C) *sul1*, and (D) *tetO* genes.

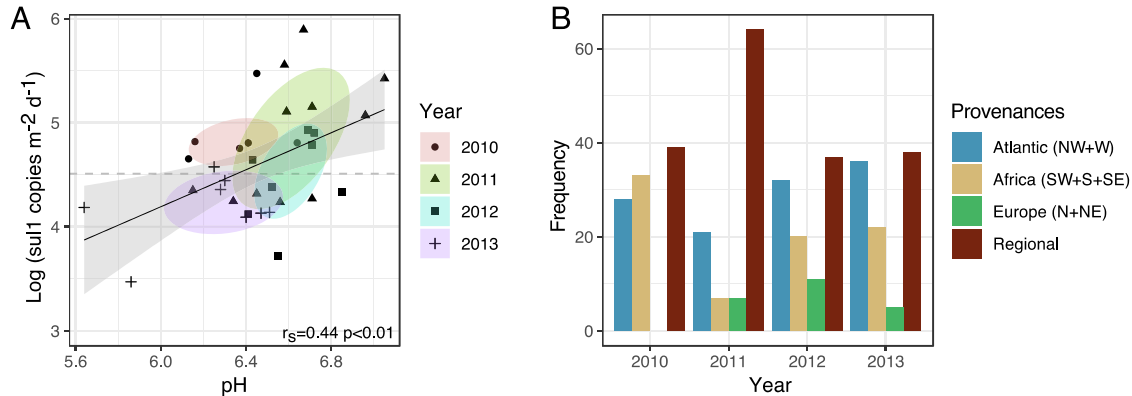


Fig. 3. (A) Relationship between *sul1* copies per m^2 per day and pH of atmospheric depositions (data from Cáliz et al. 2018). Black line: fitted linear regression; grey dashed line: median of *sul1* deposition rates. Ellipses group the samples by year at a 0.5 confidence level. (B) Frequency of back-trajectories, for all precipitation events where *sul1* was detected, associated to the main annual provenances (see also Supplementary Fig. S6).

Interestingly, we noticed that the highest *sul1* deposition rates detected in 2011 matched both precipitations dominated by regional provenances (64%, Fig. 3B and Supplementary Fig. S6) and samples with relatively higher alkalinity. Conversely, the high rates of *tetO* measured in 2012 overlapped with a exacerbated frequency of Saharan dust intrusions (Fig. 4B) and precipitations from the African continent (Supplementary Figs. S7 and S8). This fact was in agreement with the annual variations of the North Atlantic Oscillation (NAO) index, which alternated a positive and negative phase in winter and summer of 2012, respectively (inset in Fig. 4B). The behavior of the NAO index indicates dryness and increases in aerosol concentrations for Southern Europe (Jerez et al. 2013).

Positive correlations were observed between the dynamics of ARGs and selected bacterial OTUs. Bacteria with the highest positive

correlations with *int1* ($r_s > 0.50$), and *sul1* and *tetO* ($r_s > 0.35$) were mostly associated to Alpha- and Betaproteobacteria, and Bacteroidetes (Supplementary Tables S3–S5). The strongest correlations were found between *int1* (r_s range 0.70–0.84, $p < 0.001$) and the bacterial genera *Sphingomonas*, *Methylobacterium*, *Massilia* (*Oxalobacteraceae*), *Ramlibacter* and *Xylophilus* (*Comamonadaceae*) (Fig. 5A). The gene *sul1* showed the highest correlation with a *Deinococcus* ($r_s = 0.66$, $p < 0.01$), and also with *Mucilaginibacter* and *Pedobacter* (*Sphingobacteriaceae*), and *Rhodococcus* (*Corynebacteriales*) ($r_s > 0.52$, $p < 0.05$) (Fig. 5B). The gene *tetO* showed high correlations with uncultured *Acetobacteraceae*, and the genera *Tardiphaga* (*Bradyrhizobiaceae*), *Janthinobacterium* (*Oxalobacteraceae*), and *Bryocella* and *Terriglobus* (*Acidobacteriaceae*) (r_s range 0.45–0.56, $p < 0.05$) (Fig. 5C). Since a particular ARG could be hosted by different bacteria from diverse taxa, we additionally analyzed

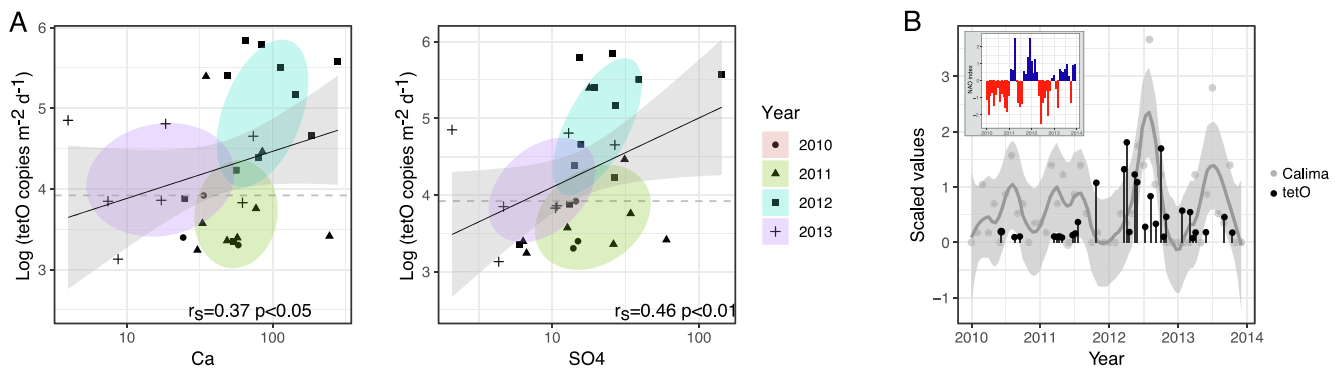


Fig. 4. (A) Relationship between *tetO* copies per m² per day, and the concentration of calcium and sulfate in atmospheric depositions (data from Cáliz et al. 2018 <http://loopweb.org/>). Black line: fitted linear regression; grey dashed line: median of ARGs deposition rates. Ellipses group samples by year at a 0.5 confidence level. (B) Number of days per month with Saharan dust intrusion (locally called *calima*) in NE Spain (data from www.calima.ws), and deposition rates of *tetO*, from 2010 to 2013. Note that values are scaled to allow the overlap of data. B inset shows the monthly oscillation of the NAO index during this period.

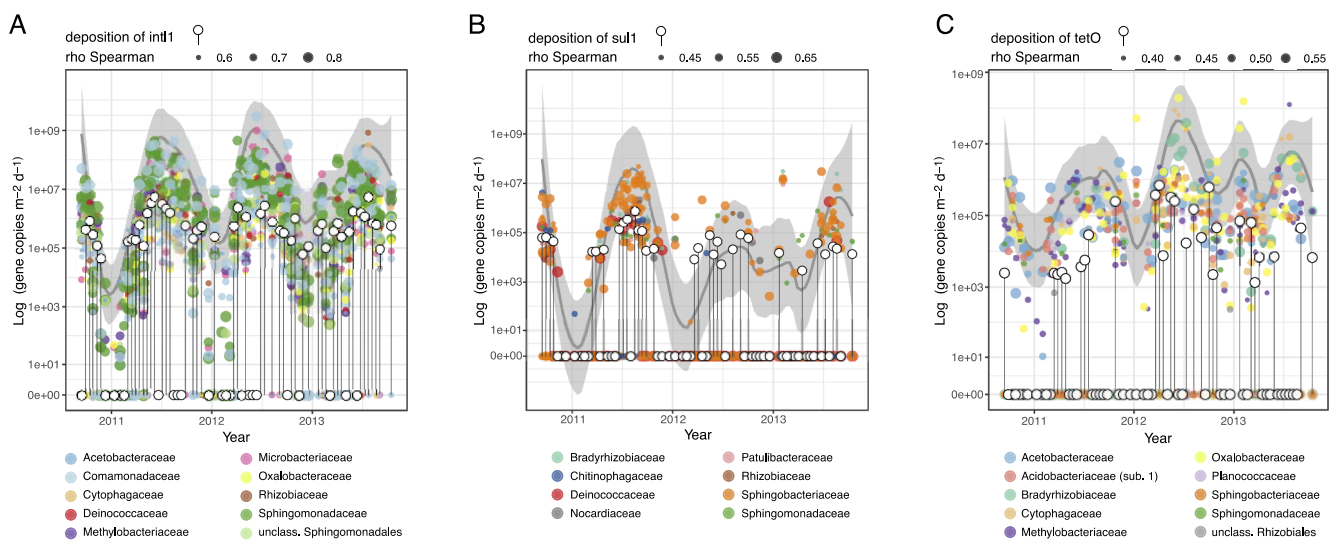


Fig. 5. Dynamics of the deposition of OTUs correlated to (A) *int11*, (B) *sul1*, and (D) *tetO*. White and colored circles represent the deposition of ARGs and OTUs, respectively. The color of the circles identifies OTUs at the family level (see legend). Temporal trend of combined deposition of all OTUs is shown by a local polynomial regression (loess: grey line).

correlations considering the sum of deposition of several OTUs at the same time, instead of individually, for each sample. Correlation coefficients greatly improved for each gene considered ($r_s = 0.89$ for *int11*, $r_s = 0.76$ for *sul1*, and $r_s = 0.73$ for *tetO*, $p < 0.001$); temporal trend is shown by local polynomial regression in Fig. 5).

Finally, for those OTUs strongly correlated with the ARGs dynamics, the most frequent sources were soil habitats (i.e. field soil, agricultural soil, cropland, pasture, forest soil) and biofilms, according to the Environmental Ontology (ENVO) terms (Supplementary Fig. S9).

4. Discussion

Globally dispersed bioaerosols connect local environments with microorganisms and genes from diverse remote origins. Our study shows that airborne ARGs from different sources have the potential to be remotely deposited overcoming geographic barriers. The log-range atmospheric dispersal of ARGs and ARB exacerbates the risk for human health and should be more carefully taken into account when designing new strategies to fight AR at both local and global scales. In this regard, any measure aimed to reduce the aerosolization of urban and rural hot-spots of resistance (e.g. hospitals, wastewater treatment plants, slurry ponds, crops) and to identify the most sensible dust upwelling zones on Earth would be welcome. In addition, the implementation of better

agricultural and livestock practices could reduce the long-range dispersal of ARGs.

4.1. Climate and air masses circulation drive atmospheric remote depositions of ARGs

Dispersal of airborne microorganisms is an issue of global concern because of increasing effects of allergens, widespread transport of pathogens, and unpredicted effects on ecosystems (Kellogg and Griffin 2006, Zhu et al. 2019, Triadó-Margarit et al. 2022). Global microbial dispersal is intimately linked to the circulation and seasonality of air masses (Cáliz et al. 2018), and transoceanic and transcontinental dust plumes injected into the atmosphere contain large concentrations of viable microorganisms and facilitate long-distance dispersal. The general atmospheric circulation of air masses is characterized by well-defined wind patterns but also influenced by local processes that shape the air microbiota. According to the current available knowledge from the IPCC reports, climate change involves alterations in mean annual air temperatures, circulation regimes of air masses, and shifts in seasonality, among others effects (<https://www.ipcc.ch/reports/>). Therefore, a major alteration can be expected in the composition, dynamics and remote depositions of the air microbiota that could be potentially anticipated and tracked (Ontiveros et al. 2021). Local

temperatures and precipitation regimes are within the main variables shaping the fate of the global air microbiome and resistome (MacFadden et al. 2018) (Cáliz et al. 2018), and these two environmental factors can be simultaneously studied. For instance, changes in the NAO signal determines changes in air temperature for Northern Europe, and a strong dependence of precipitation on the NAO index has been reported for Southern Europe (Sánchez-López et al., 2015). In the Mediterranean, the NAO is a major forcing of climate patterns, especially controlling precipitation (López-Moreno et al. 2011). The NAO effects have been extensively documented with direct or indirect impacts on terrestrial and aquatic Mediterranean ecosystems (*i.e.* plant growth and diet of herbivores, hydrodynamics in marine environments, migration of birds, among others) (Gordo et al., 2011), as well as on atmospheric pollution (Dayan, 2011).

Our study unveils relevant remote depositions of ARGs washed from the atmosphere by rain and snow beyond the atmospheric boundary layer, and that these rates were driven by the local and general air mass circulation regimes. On a global scale, the spatial distribution of ARGs is well studied for local ground surface aerosols (Li et al. 2018; Xie et al. 2018; Zhang et al. 2019; Zhou et al. 2018), and a comparative study across Europe has recently described the prevalence of ARGs in wastewater treatment plants (Pärnänen et al. 2019). We have found links between remote ARGs transport and terrestrial aerosols from southern regions following different temporal dynamics. Particularly, deposition of bacteria in summer rains increases, on average, tenfold compared to those in winter from regional and intercontinental sources. In agreement with this observation, higher deposition rates of bacteria have recently been associated with Saharan dust intrusions in contrast to marine aerosols (Reche et al. 2018), and African dust outbreaks over the Pyrenees usually have the most intense episodes during spring-summer (Salvador et al. 2014). Higher air temperatures were measured in summer too (Supplementary Table S6) that may help bacterial activity. However, we observed a direct highly significant relationship of air temperature with irradiance (that may have harmful effects on bacteria) and not significant relationship with relative humidity and, therefore, we cannot split which combination of environmental conditions maybe in favor of bacteria survival and dispersal in summer. The presence of ARGs has recently been reported in aerosols from North African sources, although the study concluded that dust storms did not seem to be an important vector for ARG transport, according to results based on normalized concentrations of ARGs by 16S rRNA copies, and to the higher abundance of atmospheric bacteria present in dust episodes as compared to dust-free days (Mazar et al. 2016). Interestingly, we observed a different deposition dynamics of tetO as compared to genes *intI1* and *sul1*, suggesting different origins and/or drivers (including bacterial hosts or atmospheric factors). We observed that aerosols carrying high amounts of tetO were enriched in calcium and sulfate, which traces an African origin (Rodríguez et al. 2011). Hence, we encourage to consider deposition rates as a more appropriate measure to assess the impact of ARG dispersal uncoupled to 16S rRNA gene dynamics. In the present study, the high deposition rates of gene tetO also matched the period in which climatic conditions favor dryness in southern regions (combination of NAO phases) and emission of Saharan dust (Rodríguez et al. 2011). Altogether, these evidences support that the dynamics of tetO can be related to the increase of African aerosols promoted by changes in climate and land uses in the region (Rodríguez et al. 2011). These findings suggests that a tight connection could be established between NAO and deposition of ARGs that deserves further research. Although we are aware of the limitation of having only one single match available between tetO and NAO, and that this fact needs further confirmation following longer temporal studies, this result emphasizes how tightly local climatic conditions drive the dynamics of aerosols and remote ARGs depositions. The current climate change scenario, the increase of deforestation, and transformations of Earth's surface and land uses, all in all combined with the lack of strict regulations in underdeveloped countries, exacerbate the global dispersal of antibiotic

resistances and more integral responses are needed.

4.2. Sources of airborne antibiotic resistance bacteria

Antibiotic resistances have largely increased in the last decades due to the selection pressure that antibiotic residues exert on aquatic and terrestrial ecosystems with effects at local and global scales (Hernando-Amado et al. 2019). ARB and ARGs are present in a large variety of sources such as wastewater discharges from human settlements (*e.g.* hospitals, urban areas) or animal manure used for crop fertilization (Bougnom and Piddock 2017; Zhu et al. 2019). The growing interest in airborne AR (Li et al. 2018; Zhang et al. 2019) mainly arises from the potential harmful effects on human and planetary health (Ben et al. 2019; Zhu et al. 2019). Local aerosols from hospital, animal husbandry, and wastewater treatment plants are known to contain ARB (Chapin et al. 2005; Gao et al. 2014; Gibbs et al. 2006; Teixeira et al. 2016). Common AR pathogens are frequently isolated from local air samples such as *Acinetobacter baumannii*, *Enterococcus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Escherichia coli* and *Enterobacter cloacae*, among others. Remarkably, most of these targets were identified in our tropospheric survey as reported in detail elsewhere (Triadó-Margarit et al. 2022), although both their deposition dynamics and ARGs dynamics were uncoupled (data not shown). Hence, we conclude that most probably these taxa were not the main long-range/long-term vectors for the ARGs that we measured in the high mountain aerosols. Conversely, we found additional ARB with similar depositions dynamics to those found for ARGs that have been reported to grow in presence of antibiotics such as *Sphingomonas*, *Mucilaginibacter* or *Methylobacterium* (Baik et al. 2010; Jourand et al. 2004; Vaz-Moreira et al. 2011) and, interestingly, most of them are common inhabitants of soil, particularly agricultural environments and croplands. In fact, tetracycline resistant bacteria isolated from agricultural soils (Pérez-Valera et al. 2019) matched with some of the airborne bacteria identified in our study (*e.g.* *Rizhobium*, *Rhodococcus*). Tight potential connections can be therefore established between the tropospheric ARG signals and the soil resistome. The composition and abundance of the soil resistome is shaped by the different crop management practices, regime of manure amendments, and antibiotic load practices in animal husbandry (Dungan et al. 2019). Agricultural management using manure or sludge compost as fertilizer exacerbates the enrichment and dissemination of the soil resistome and can lead to substantial differences in ARGs composition in soils as compared with other sources (Zhu et al. 2019). These differences could potentially explain why we remotely detected airborne genes encoding resistance to *sul1* and *tetO* (belonging to sulfonamides and tetracyclines families, common in veterinary and animal husbandry), but not to *bla-TEM* or *bla-CTX-M* and *ermB* (belonging to β -lactams and macrolides families, more common in human waste). Again, this observation agrees with soils (and more likely agricultural soils) being the most relevant potential source of airborne ARGs. These findings are also supported by a recent investigation concluding that agricultural activity exerts a strong influence to local dryland aerobiomes which locally disseminate potential pathogens and ARGs (Finn et al. 2021). The vast agricultural surface on Earth can generate a large amount of aerosols, especially during summer droughts and under specific combinations of NAO phases in Mediterranean and North African areas. We therefore call for a more careful evaluation of the tight connections between agricultural soils practices and atmospheric long-range/long-term ARB dispersal that deserve further investigations.

Overall, our study highlights that the remote deposition of airborne ARGs has closer links with terrestrial than with oceanic sources. The specific origin and trajectory of air masses regulate the intensity and composition of remote ARGs depositions. The source-to-air exchange capacity, determined by both climatic patterns and idiosyncrasy of resistance reservoirs (*e.g.* dryness, exposition to wind and convection, antibiotic and waste management practices), may also shape the long-range dispersal of ARGs. Global changes anomalies may exacerbate

ARB interactions between the atmosphere, hydrosphere, biosphere, lithosphere, and anthroposphere compartments threatening the Global-Health (Hernando-Amado et al. 2019). Our findings highlight that regular and predictable depositions of ARGs should be expected under giving climatic scenarios with well-defined weather fluctuations and atmospheric circulation regimens. Difficulties are higher however, to foresee how climate warming and accelerated global transformations will reshape the circulation of air masses, the intensity and frequency of aerosols emissions and precipitation regimes, and how these changes will eventually modify global ARGs dispersal dynamics.

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CRedit authorship contribution statement

Joan Cáliz: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Jessica Subirats:** Data curation, Formal analysis, Investigation, Methodology. **Xavier Triadó-Margarit:** Data curation, Formal analysis, Investigation, Methodology. **Carles M. Borrego:** Conceptualization, Funding acquisition, Investigation, Supervision, Writing - original draft. **Emilio O. Casamayor:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Writing - review & editing.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2022.107077>. qPCR and chemical data are available as Supplementary information. The 16S rRNA gene datasets are available in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) project PRJEB14358. Meteorological data is available at <http://loop-web.org/>.

References

Allen, H.K., Donato, J., Wang, H.H., Cloud-Hansen, K.A., Davies, J., Handelsman, J.O., 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nat Rev Micro* 8 (4), 251–259.

- Baik, K.S., Park, S.C., Kim, E.M., Lim, C.H., Seong, C.N., 2010. Mucilaginibacter rigui sp. nov., isolated from wetland freshwater, and emended description of the genus Mucilaginibacter. *Int. J. Syst. Evol. Microbiol.* 60, 134–139.
- Ben, Y., Fu, C., Hu, M., Liu, L., Wong, M.H., Zheng, C., 2019. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. *Environ. Res.* 169, 483–493.
- Bougnom, B.P., Piddock, L.J.V., 2017. Wastewater for Urban Agriculture: A Significant Factor in Dissemination of Antibiotic Resistance. *Environ. Sci. Technol.* 51 (11), 5863–5864.
- Cáliz, J., Triadó-Margarit, X., Camarero, L., Casamayor, E.O., 2018. A long-term survey unveils strong seasonal patterns in the airborne microbiome coupled to general and regional atmospheric circulations. *Proc. Natl. Acad. Sci.* 115 (48), 12229–12234.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, J., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Meth.* 7 (5), 335–336.
- Chapin, A., Rule, A., Gibson, K., Buckley, T., Schwab, K., 2005. Airborne Multidrug-Resistant Bacteria Isolated from a Concentrated Swine Feeding Operation. *Environ. Health Perspect.* 113 (2), 137–142.
- Collignon, P., Beggs, J.J., Walsh, T.R., Gandra, S., Laxminarayan, R., 2018. Anthropological and socioeconomic factors contributing to global antimicrobial resistance: a univariate and multivariable analysis. *Lancet Planetary Health* 2 (9), e398–e405.
- D'Costa, V.M., King, C.E., Kalan, L., Morar, M., Sung, W.W.L., Schwarz, C., Froese, D., Zazula, G., Cabelm, F., Debruyne, R., Golding, G.B., Poinar, H.N., Wright, G.D., 2011. Antibiotic resistance is ancient. *Nature* 477 (7365), 457–461.
- D'Costa, V.M., McGrann, K.M., Hughes, D.W., Wright, G.D., 2006. Sampling the Antibiotic Resistome. *Science* 311 (5759), 374–377.
- Davies, J., Davies, D., 2010. Origins and Evolution of Antibiotic Resistance. *Microbiology and Molecular Biology Reviews* : MMBR 74 (3), 417–433.
- Dayan, U. Impacts of the NAO on Atmospheric Pollution in the Mediterranean Basin. In: Vicente-Serrano S., Trigo R. (eds) Hydrological, Socioeconomic and Ecological Impacts of the North Atlantic Oscillation in the Mediterranean Region. *Advances in Global Change Research*, vol 46. ed's: Springer, Dordrecht; 2011.
- Després, VivianeR., Huffman, J.A., Burrows, S.M., Hoose, C., Safatov, AleksandrS., Buryak, G., Fröhlich-Nowoisky, J., Elbert, W., Andreae, MeinratO., Pöschl, U., Jaenicke, R., 2012. Primary biological aerosol particles in the atmosphere: a review. *Tellus B: Chem. Phys. Meteorol.* 64 (1), 15598. <https://doi.org/10.3402/tellusb.64i0.15598>.
- Dungan, R.S., Strausbaugh, C.A., Leytem, A.B., 2019. Survey of selected antibiotic resistance genes in agricultural and non-agricultural soils in south-central Idaho. *FEMS Microbiol. Ecol.* 95 (6) <https://doi.org/10.1093/femsec/fiz071>.
- Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Meth.* 10 (10), 996–998.
- Els, N., Larose, C., Baumann-Stanzer, K., Tignat-Perrier, R., Keusch, N., Vogel, T.M., Sattler, B., 2019. Microbial composition in seasonal time series of free tropospheric air and precipitation reveals community separation. *Aerobiologia* 35 (4), 671–701.
- Finn, D.R., Maldonado, J., de Martini, F., Yu, J., Penton, C.R., Fontenele, R.S., Schmidlin, K., Kraberger, S., Varsani, A., Gile, G.H., Barker, B., Kollath, D.R., Muenich, R.L., Herckes, P., Fraser, M., Garcia-Pichel, F., 2021. Agricultural practices drive biological loads, seasonal patterns and potential pathogens in the aerobiome of a mixed-land-use dryland. *Sci. Total Environ.* 798, 149239. <https://doi.org/10.1016/j.scitotenv.2021.149239>.
- Forsberg, K.J., Patel, S., Gibson, M.K., Lauber, C.L., Knight, R., Fierer, N., Dantas, G., 2014. Bacterial phylogeny structures soil resistomes across habitats. *Nature* 509 (7502), 612–616.
- Forsberg, K.J., Reyes, A., Wang, B., Selleck, E.M., Sommer, M.O.A., Dantas, G., 2012. The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens. *Science* 337 (6098), 1107–1111.
- Gao, J., Zhao, X., Bao, Y., Ma, R., Zhou, Y., Li, X., Chai, T., Cai, Y., 2014. Antibiotic resistance and OXA-type carbapenemase-encoding genes in airborne Acinetobacter baumannii isolated from burn wards. *Burns* 40 (2), 295–299.
- Gao, M., Qiu, T., Sun, Y., Wang, X., 2018. The abundance and diversity of antibiotic resistance genes in the atmospheric environment of composting plants. *Environ. Int.* 116, 229–238.
- Gibbs, S.G., Green, C.F., Tarwater, P.M., Mota, L.C., Mena, K.D., Scarpino, P.V., 2006. Isolation of Antibiotic-Resistant Bacteria from the Air Plume Downwind of a Swine Confined or Concentrated Animal Feeding Operation. *Environ. Health Perspect.* 114 (7), 1032–1037.
- Gillings, M.R., Gaze, W.H., Pruden, A., Smalla, K., Tiedje, J.M., Zhu, Y.-G., 2015. Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. *ISME J* 9 (6), 1269–1279.
- Goossens, H., Ferech, M., Vander Stichele, R., Elseviers, M., 2005. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *The Lancet* 365 (9459), 579–587.
- Gordo, O.; Barriocanal, C.; Robson, D. Ecological Impacts of the North Atlantic Oscillation (NAO) in Mediterranean Ecosystems. In: Vicente-Serrano S., Trigo R. (eds) Hydrological, Socioeconomic and Ecological Impacts of the North Atlantic Oscillation in the Mediterranean Region. *Advances in Global Change Research*, vol 46. ed's: Springer, Dordrecht; 2011.
- Hernando-Amado, S., Coque, T.M., Baquero, F., Martínez, J.L., 2019. Defining and combating antibiotic resistance from One Health and Global Health perspectives. *Nat. Microbiol.* 4 (9), 1432–1442.

- Hervás, A., Camarero, L., Reche, I., Casamayor, E.O., 2009. Viability and potential for immigration of airborne bacteria from Africa that reach high mountain lakes in Europe. *Environ. Microbiol.* 11 (6), 1612–1623.
- Izquierdo, R., Avila, A., Alarcón, M., 2012. Trajectory statistical analysis of atmospheric transport patterns and trends in precipitation chemistry of a rural site in NE Spain in 1984–2009. *Atmos. Environ.* 61, 400–408.
- Jerez, S., Jimenez-Guerrero, P., Montávez, J.P., Trigo, R.M., 2013. Impact of the North Atlantic Oscillation on European aerosol ground levels through local processes: a seasonal model-based assessment using fixed anthropogenic emissions. *Atmos. Chem. Phys.* 13 (22), 11195–11207.
- Jourand, P., Giraud, E., Béna, G., Sy, A., Willems, A., Gillis, M., Dreyfus, B., de Lajudie, P., 2004. *Methylobacterium nodulans* sp. nov., for a group of aerobic, facultatively methylotrophic, legume root-nodule-forming and nitrogen-fixing bacteria. *Int. J. Syst. Evol. Microbiol.* 54, 2269–2273.
- Kaba, H.E.J., Kuhlmann, E., Scheithauer, S., 2020. Thinking outside the box: Association of antimicrobial resistance with climate warming in Europe – A 30 country observational study. *Int. J. Hyg. Environ. Health* 223 (1), 151–158.
- Kellogg, C.A., Griffin, D.W., 2006. Aerobiology and the global transport of desert dust. *Trends Ecol. Evol.* 21 (11), 638–644.
- Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia* 129 (2), 271–280.
- Li, J., Cao, J., Zhu, Y.-G., Chen, Q.-L., Shen, F., Wu, Y., Xu, S., Fan, H., Da, G., Huang, R.-J., Wang, J., de Jesus, A.L., Morawska, L., Chan, C.K., Peccia, J., Yao, M., 2018. Global Survey of Antibiotic Resistance Genes in Air. *Environ. Sci. Technol.* 52 (19), 10975–10984.
- Li, J., Zhou, L., Zhang, X., Xu, C., Dong, L., Yao, M., 2016. Bioaerosol emissions and detection of airborne antibiotic resistance genes from a wastewater treatment plant. *Atmos. Environ.* 124, 404–412.
- López-Moreno, J.I.; Vicente-Serrano, S.M.; Morán-Tejada, E.; Lorenzo-Lacruz, J.; Zabalza, J.; Kenawy, A.E.; Beniston, M. Influence of Winter North Atlantic Oscillation Index (NAO) on Climate and Snow Accumulation in the Mediterranean Mountains. In: Vicente-Serrano S., Trigo R. (eds) *Hydrological, Socioeconomic and Ecological Impacts of the North Atlantic Oscillation in the Mediterranean Region*. *Advances in Global Change Research*, vol 46. ed's: Springer, Dordrecht; 2011.
- MacFadden, D.R., McGough, S.F., Fisman, D., Santillana, M., Brownstein, J.S., 2018. Antibiotic resistance increases with local temperature. *Nat. Clim. Change* 8 (6), 510–514.
- Mazar, Y., Cytryn, E., Erel, Y., Rudich, Y., 2016. Effect of Dust Storms on the Atmospheric Microbiome in the Eastern Mediterranean. *Environ. Sci. Technol.* 50 (8), 4194–4202.
- McKenna, M., 2013. Antibiotic resistance: the last resort. *Nature* 499 (7459), 394–396.
- Ontiveros, V.J., Cáliz, J., Triadó-Margarit, X., Alonso, D., Casamayor, E.O., 2021. General decline in the diversity of the airborne microbiota under future climatic scenarios. *Sci Rep* 11, 20223. <https://doi.org/10.1038/s41598-021-99223-x>.
- Oksanen, J.; Blanchet, F.G.; Kindt, R.; Legendre, P.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; Stevens, M.H.H.; Wagner, H. *vegan: Community Ecology Package*. R package version 2.0-10. 2013.
- Pärnänen, K.M.M., Narciso-da-Rocha, C., Kneis, D., Berendonk, T.U., Cacace, D., Do, T.T., Elpers, C., Fatta-Kassinos, D., Henriques, I., Jaeger, T., Karkman, A., Martinez, J.L., Michael, S.G., Michael-Kordatou, I., O'Sullivan, K., Rodriguez-Mozaz, S., Schwartz, T., Sheng, H., Sörum, H., Stedtfeld, R.D., Tiedje, J.M., Giustina, S.V.D., Walsh, F., Vaz-Moreira, I., Virta, M., Manaia, C.M., 2019. Antibiotic resistance in European wastewater treatment plants mirrors the pattern of clinical antibiotic resistance prevalence. *Sci. Adv.* 5 (3) <https://doi.org/10.1126/sciadv.aau9124>.
- Pérez-Valera, E., Kyselková, M., Ahmed, E., Sladeczek, F.X.J., Goberna, M., Elhottová, D., 2019. Native soil microorganisms hinder the soil enrichment with antibiotic resistance genes following manure applications. *Sci. Rep.* 9, 6760.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glockner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* 41, D590–596.
- Reche, I., D'Orta, G., Mladenov, N., Winget, D.M., Suttle, C.A., 2018. Deposition rates of viruses and bacteria above the atmospheric boundary layer. *ISME J.* 12 (4), 1154–1162.
- Rodríguez, S., Alastuey, A., Alonso-Pérez, S., Querol, X., Cuevas, E., Abreu-Afonso, J., Viana, M., Pérez, N., Pandolfi, M., de la Rosa, J., 2011. Transport of desert dust mixed with North African industrial pollutants in the subtropical Saharan Air Layer. *Atmos. Chem. Phys.* 11 (13), 6663–6685.
- Salvador, P., Alonso-Pérez, S., Pey, J., Artíñano, B., de Bustos, J.J., Alastuey, A., Querol, X., 2014. African dust outbreaks over the western Mediterranean Basin: 11-year characterization of atmospheric circulation patterns and dust source areas. *Atmos. Chem. Phys.* 14 (13), 6759–6775.
- Sánchez-López, G., Hernández, A., Pla-Rabes, S., Toro, M., Granados, I., Sigró, J., Trigo, R.M., Rubio-Inglés, M.J., Camarero, L., Valero-Garcés, B., Giral, S., 2015. The effects of the NAO on the ice phenology of Spanish alpine lakes. *Clim. Change* 130 (2), 101–113.
- Sinclair, L., Ijaz, U.Z., Jensen, L.J., Coolen, M.J.L., Gubry-Rangin, C., Chroňáková, A., Oulas, A., Pavlou, C., Schnetzer, J., Weimann, A., Ijaz, A., Eiler, A., Quince, C., Pafilis, E., 2016. Seqenv: linking sequences to environments through text mining. *PeerJ* 4, e2690.
- Smillie, C.S., Smith, M.B., Friedman, J., Cordero, O.X., David, L.A., Alm, E.J., 2011. Ecology drives a global network of gene exchange connecting the human microbiome. *Nature* 480 (7376), 241–244.
- Smith, D.J., Timonen, H.J., Jaffe, D.A., Griffin, D.W., Birmele, M.N., Perry, K.D., Ward, P. D., Roberts, M.S., 2013. Intercontinental Dispersal of Bacteria and Archaea by Transpacific Winds. *Appl. Environ. Microbiol.* 79 (4), 1134–1139.
- Subirats, J., Royo, E., Balcázar, J.L., Borrego, C.M., 2017. Real-time PCR assays for the detection and quantification of carbapenemase genes (bla KPC, bla NDM, and bla OXA-48) in environmental samples. *Environ. Sci. Pollut. Res.* 24 (7), 6710–6714.
- Teixeira, J.V., Cecílio, P., Gonçalves, D., Vilar, V.J.P., Pinto, E., Ferreira, H.N., 2016. Multidrug-resistant Enterobacteriaceae from indoor air of an urban wastewater treatment plant. *Environ. Monit. Assess.* 188, 388.
- Triadó-Margarit, X., Caliz, J., Reche, I., Casamayor, E.O., 2019. High similarity in bacterial bioaerosol compositions between the free troposphere and atmospheric depositions collected at high-elevation mountains. *Atmos. Environ.* 203, 79–86.
- Triadó-Margarit, X., Cáliz, J., Casamayor, E.O., 2022. A long-term atmospheric baseline for intercontinental exchange of airborne pathogens. *Environ. Int.* 158, 106916. <https://doi.org/10.1016/j.envint.2021.106916>.
- Vaz-Moreira, I., Nunes, O.C., Manaia, C.M., 2011. Diversity and Antibiotic Resistance Patterns of Sphingomonadaceae Isolates from Drinking Water. *Appl. Environ. Microbiol.* 77, 5697–5706.
- Vaz-Moreira, I., Nunes, O.C., Manaia, C.M., 2014. Bacterial diversity and antibiotic resistance in water habitats: searching the links with the human microbiome. *FEMS Microbiol. Rev.* 38 (4), 761–778.
- Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73.
- Wickham, H. (Ed.), 2009. *ggplot2*. Springer New York, New York, NY.
- Xie, J., Jin, L., Luo, X., Zhao, Z., Li, X., 2018. Seasonal Disparities in Airborne Bacteria and Associated Antibiotic Resistance Genes in PM2.5 between Urban and Rural Sites. *Environ. Sci. Technol. Lett.* 5 (2), 74–79.
- Zhang, T., Li, X., Wang, M., Chen, H., Yang, Y., Chen, Q.-L., Yao, M., 2019. Time-resolved spread of antibiotic resistance genes in highly polluted air. *Environ. Int.* 127, 333–339.
- Zhou, H., Wang, X., Li, Z., Kuang, Y.u., Mao, D., Luo, Y.i., 2018. Occurrence and Distribution of Urban Dust-Associated Bacterial Antibiotic Resistance in Northern China. *Environ. Sci. Technol. Lett.* 5 (2), 50–55.
- Zhu, Y.-G., Johnson, T.A., Su, J.-Q., Qiao, M., Guo, G.-X., Stedtfeld, R.D., Hashsham, S.A., Tiedje, J.M., 2013. Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proc. Natl. Acad. Sci.* 110 (9), 3435–3440.
- Zhu, Y.-G., Zhao, Y.i., Zhu, D., Gillings, M., Penuelas, J., Ok, Y.S., Capon, A., Banwart, S., 2019. Soil biota, antimicrobial resistance and planetary health. *Environ. Int.* 131, 105059. <https://doi.org/10.1016/j.envint.2019.105059>.