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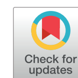
Contribution of soil bacteria to the atmosphere across biomes



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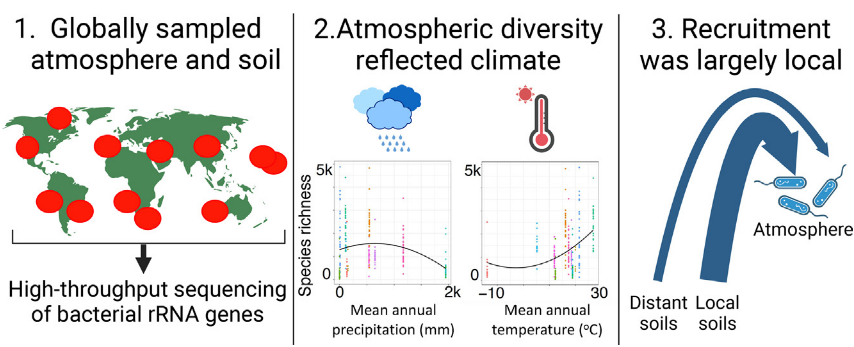
HIGHLIGHTS

- Bacterial diversity in the atmosphere reflected strong environmental filtering.
- Observed species richness was correlated with macroclimate on a global scale.
- Local soils contributed more than distant soils to atmospheric bacterial diversity.

GRAPHICAL ABSTRACT



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ABSTRACT

The dispersion of microorganisms through the atmosphere is a continual and essential process that underpins biogeography and ecosystem development and function. Despite the ubiquity of atmospheric microorganisms globally, specific knowledge of the determinants of atmospheric microbial diversity at any given location remains unresolved. Here we describe bacterial diversity in the atmospheric boundary layer and underlying soil at twelve globally distributed locations encompassing all major biomes, and characterise the contribution of local and distant soils to the observed atmospheric community. Across biomes the diversity of bacteria in the atmosphere was negatively correlated with mean annual precipitation but positively correlated to mean annual temperature. We identified distinct non-randomly assembled atmosphere and soil communities from each location, and some broad trends persisted across biomes including the enrichment of desiccation and UV tolerant taxa in the atmospheric community. Source tracking revealed that local soils were more influential than distant soil sources in determining observed diversity in the atmosphere, with more emissive semi-arid and arid biomes contributing most to signatures from distant soil. Our findings highlight complexities in the atmospheric microbiota that are relevant to understanding regional and global ecosystem connectivity.

Abbreviations

ABL	Atmospheric boundary layer
MAP	mean annual precipitation
MAT	mean annual temperature

1. Introduction

The atmospheric boundary layer (ABL) comprises the lowermost well-mixed part of the troposphere, which supports diverse microorganisms that are continuously aerosolised and dispersed from surface source habitats (Šantl-Temkiv et al., 2022). As an airmass transits above various habitats, new taxa will be added and their airborne longevity will depend on a suite of factors such as cell size, association with particles, wind speed (Burrows et al., 2009a). Taxa aerosolized from the closest source are expected to dominate the microbial community, particularly for labile surfaces or high biomass soils (Tignat-Perrier et al., 2019). There have been attempts to track these sources (Lymperopoulou et al., 2016; Mayol et al., 2017), however the fundamental question of how significant the contributions of local and distant soils are to the ABL microbiota remains largely unanswered.

At any location, the biomass of microorganisms in the ABL is extremely low, with values ranging from 10^1 to 10^8 cells/m^3 for bacteria and from undetectable to 10^5 cells/m^3 for fungi (Tignat-Perrier et al., 2019), whilst hotspots of microbial abundance and diversity occur in clouds (Amato et al., 2007), and particulate plumes from desert dust (Maki et al., 2017) and wildfires (Kobziar et al., 2022). Recent advances in methodology for the study of ultra-low biomass microbiomes (Eisenhofer et al., 2019; Luhung et al., 2021; Šantl-Temkiv et al., 2020), have led to enhanced understanding of microbial diversity of the ABL at several locations. A number of recent studies have assessed spatial and temporal changes in ABL microbial communities and concluded that variation reflected passage of air mass trajectories above land or ocean with different uses (Archer et al., 2020; Caliz et al., 2019; Els et al., 2019; Lang-Yona et al., 2022; Tignat-Perrier et al., 2019). Others have identified local land use as a potential factor influencing microbial communities, e.g., (Gusareva et al., 2019; Lymperopoulou et al., 2016; Nicolaisen et al., 2017; Spring et al., 2021). Studies of remote terrestrial (Archer et al., 2019) and marine (Uetake et al., 2020) locations have revealed that atmospheric transport limitation can result in biogeographic patterns for bacteria and fungi in the ABL. Considerable geographic knowledge gaps exist regarding microbial communities in the ABL, and particularly for natural dryland biomes that support highly emissive soils, and studies that have directly compared atmospheric microbiota across biomes are scarce (Tignat-Perrier et al., 2019; Zhao et al., 2022). Some studies have applied source-tracking algorithms to identify potential origin of bacteria in the ABL, e.g., (Mu et al., 2020; Uetake et al., 2019), although a limitation has been the lack of relevant surface microbial datasets with which to compare data from atmospheric samples. A major knowledge gap therefore arises where data is lacking for concurrently sampled atmospheric and underlying soil communities across different biomes.

To further advance understanding regarding the relationship between bacterial diversity in the ABL and surface biomes, we concurrently sampled the ABL and soil at twelve locations across six continents that encompassed all major climate categories. We used 16S rRNA gene amplicon sequencing with a rigorous process to remove potential contaminants that are encountered in low biomass samples (Eisenhofer et al., 2019; Salter et al., 2014), to characterise bacterial diversity. We estimated the influence of macroclimate factors on observed diversity, modelled the assembly of communities, and employed the FEAST source-tracking algorithm to identify the contribution of local and non-local soil communities to the atmospheric boundary layer. Our findings provide a globally distributed baseline reference dataset for application in future efforts to further resolve the sources of atmospheric microorganisms.

2. Methods

2.1. Sample recovery

The sampling campaign retrieved 469 ABL and soil samples from 12 locations globally (Table 1). The Southern Hemisphere (Australia, South Africa, Namibia, Uruguay and Chile) was sampled during April–May 2019 and the Northern Hemisphere (Hawaii (Hilo and Mauna Kea), California, Arctic, Spain, Kuwait, Mongolia) during June–July 2019. All samples had negative field sampling controls for each day of sampling to test for potential contamination of equipment and field consumables, and additional negative laboratory controls for each of the sample-randomised DNA extraction batches to identify any potential contamination introduced throughout sampling and laboratory workflow. All control samples were processed as per experimental samples.

Atmospheric particles, including microbial cells, were sampled from the ABL using three Coriolis μ high-volume impingement devices (Bertin Instruments) operated concurrently. All equipment was transported between locations in sterile containers and bags. Each device was dis-assembled and contact surfaces soaked for 1 h with 1.5 % *v*/v sodium hypochlorite (NaClO) followed by three washes of Milli-Q H_20 prior to and after each sampling in order minimise contamination from cells or nucleic acids. All apparatus and work surfaces used during sampling and sample processing were also cleaned in this way prior to use. All operators wore surface sterilised nitrile gloves during field collections. Air samplers were located on tripods 1.5 m above the ground and 3 m apart from each other at each sampling location and all inlets were aligned facing prevalent local wind direction. Samplers were only approached from downwind during operation.

Atmospheric particles were collected in 18 m^3 volumes (300 L/min^{-1} for 1 h) into a sterile phosphate-buffered saline (PBS) impingement medium as this volume has been shown to result in recoverable environmental DNA (Archer et al., 2019). Sampling cones were replaced at hourly intervals during a standardised sampling window ±3 h from solar noon at each location. For sampling blanks at each location, collection cones were assembled into the devices for 1 min without activating the air pump, and these were used as the negative sampling controls at each location. Collected samples were further concentrated by syringe filtration onto 25 mm polycarbonate filters with 0.2 μm pore size and preserved in 0.5 mL of DNA/RNA Shield (Zymo Research, USA) at ambient temperature during transit and then frozen at −20 °C until processed for DNA extraction in the laboratory.

At each location 6 undisturbed surface soil samples (upper 2 cm soil captured in sterile 50 mL screw-cap tubes) were collected. One was collected from the base of each Coriolis m device and three were collected each 25 m away in different evenly spaced directions. In recognition of inherent soil heterogeneity each sample comprised five subsamples that were mixed and then resampled to yield a representative sample for each analysis. For each sample 0.5 g was preserved for DNA extraction in 0.5 mL of DNA/RNA Shield (Zymo Research, USA) at ambient temperature during transit and then frozen at −20 °C until processed for DNA extraction in the laboratory.

Environmental DNA was recovered from filtered atmosphere and soil samples using a CTAB-based manual extraction protocol optimised for low biomass samples (Archer et al., 2019). DNA extractions from samples were performed under strict microbiological biosafety conditions in randomised sample batches, and each batch was processed with discreet laboratory controls to assess potential laboratory or reagent contamination. Each sample tube was processed individually to avoid potential cross-contamination between samples due to micro-droplet transfer. DNA yield was quantified using the Qubit 2.0 Fluorometer (Invitrogen, USA) and samples were then stored at −20 °C until processed.

2.2. Biomass estimation

Biomass estimation was made using a well-established real-time quantitative PCR (qPCR) approach (Hospodsky et al., 2010). Primers used for bacteria were S-D-Bact-0341-b-S-17 (5′-CCTACGGGNGGCWGCAG-3′) and D-Bact-0785-a-A-21 (5′-GACTACHVGGGTATCTAATCC-3′) (Herlemann et al., 2011; Klindworth et al., 2013) with LightCycler 480 SYBR Green I Master mix (Roche Holding, Switzerland). A qPCR standard for the target sequence was developed to estimate gene copy number using pooled samples of all extracted atmosphere and soil samples. These were amplified using TaqMan Fast Advanced Master Mix without fluorescent markers (Applied biosystems, USA) with the following conditions: denaturing step: 95 °C for 20 s; cycling step: 35 cycles of 95 °C for 1 s and 60 °C for 20 s as used previously (Gusareva et al., 2019). These were then quantified using a Bioanalyzer (Agilent Technologies, USA). Serial dilution of the DNA was used to generate standard curves.

2.3. DNA sequencing

Sequence libraries were prepared using Illumina MiSeq v3 600 cycle chemistry as per manufacturer's protocol. Template DNA in samples was normalised prior to two-step PCR amplification for the bacterial 16S rRNA gene V3-V4 hypervariable region (Herlemann et al., 2011; Klindworth et al., 2013) as previously described (Archer et al., 2019). Libraries were first processed with cutadapt v2.7 (Martin, 2011) to remove primer sequences and amplicon sequence variants (ASVs) (Callahan et al., 2017), were generated using dada2 v1.14 (Callahan et al., 2016). Pseudo-pooling was used in ASV calling to increase sensitivity and detect rare ASVs. Taxonomic classification was conducted in dada2 with the SILVA v138 database (Quast et al., 2013). DNA concentration for all control samples were below detection limits but these samples were retained through amplicon sequencing preparation and run with associated samples. Any samples associated with controls gaining >500 amplicon reads were removed from downstream analysis. Decontamination was conducted using the R package decontam (Davis et al., 2018) on all remaining samples and controls resulting in 96.55 % of reads being retained. The decontamination process was conducted by grouping samples into batches with corresponding sampling controls from the same location and extraction controls from the same sequencing batch. Potential contaminant ASVs were identified using the "isContaminant" function with the default "combine" method, with a default probability threshold of 0.1 and the default "minimum" method for combining the *p*-values of the ASVs across batches. In a given batch, the p-value of an ASV not being a contaminant (the null hypothesis) based on the frequency and prevalence methods were combined using Fisher's method. The lowest p-value of a given ASV across batches was used. Any ASV with a final p-value < 0.1 was suspected to be a contaminant and removed. Any samples with >15 % of reads removed or with <1000 reads remaining were removed to ensure reliable representation of community structures resulting in a total of 309 biological samples for diversity analysis (Table 1). Taxonomic identity of reads removed from retained samples during the decontamination process is reported according to recommended best practice (Eisenhofer et al., 2019) (Appendix A). Sampling curves for all post-filtered atmosphere and soil samples achieved near-asymptote for ASV diversity (Appendix A). Sequence data for all samples and controls are accessible in the European Nucleotide Archive at EMBL-

Table 1
Globally distributed sampling locations included in this study. Locations are ordered by latitude from north to south. Macroclimate data were obtained from long-term observations (https://climatecharts.net). Climate codes follow the Köppen climate classification: major delineations were: A, tropical; B, dry; C, temperate; D, continental, E, polar [Peel MC, Finlayson BL & McMahon TA (2007), Updated world map of the Köppen-Geiger climate classification, Hydrol. Syst. Sci. 11,1633–1644]. Number of samples retained for downstream analysis after decontamination are shown for atmosphere (Atm.) and soil.

Location	GPS (decimal degrees)	MAP (mean annual precipitation, mm)	MAT (mean annual temperature, °C)	Altitude (m above mean sea level)	Climate	Biome (surface cover)	No. samples	
							Atm.	Soil
Arctic	69.131, −105.057	136.1	−13.9	50	Polar (Ef)	Polar ice (severe tundra)	12	5
Mongolia	44.573, 105.648	116.5	5.2	1235	Dry (BWk)	Cold arid desert (grassland)	12	6
Spain	40.825, −3.961	537.8	11.9	1830	Continental (Dsb)	Warm summer hemiboreal (grassland)	26	5
California, USA	35.142, −116.104	153.1	20.0	300	Dry (BWh)	Hot arid desert (Mojave Desert)	5	6
Kuwait	28.951, 48.192	109.9	26.9	0	Dry (BWh)	Hot arid desert (coastal desert)	25	6
Hilo, Hawaii, USA	19.703, −155.090	1949.3	18.9	120	Tropical (Af)	Tropical rainforest (coastal)	6	1
Mauna Kea, Hawaii, USA	19.823, −155.478	1949.3	18.9	4200	Polar (Et)	Tundra (mountain peak)	27	6
Namibia	−23.603, 15.038	10.4	21.5	380	Dry (BWh)	Hot arid desert (Namib Desert)	29	5
Chile	−24.105, −70.016	1	12.3	90	Dry (BWk)	Cold arid desert (Atacama Desert)	28	4
South Africa	−25.753, −28.258	648.9	18.5	1380	Temperate (Cwb)	Subtropical highland (livestock and arable)	16	6
Australia	−32.898, 116.906	544.3	16.2	320	Temperate (Csa)	Mediterranean hot summer (grassland and arable)	29	5
Uruguay	−34.354, −57.235	1168.7	17.4	10	Temperate (Cfa)	Humid subtropical (wooded grassland)	33	6

EBI under project accession number PRJEB58182 (https://www.ebi.ac.uk/ena/browser/view/PRJEB58182).

2.4. Statistical treatments and ecological modelling

General processing of bacterial ASV data including the calculation of relative abundance, estimates of alpha and beta diversity, and significance testing were conducted using the R package phyloseq (McMurdie and Holmes, 2013) and visualised using ggplot2 (Wickham, 2009). To visualize patterns of community dissimilarity, Hellinger distances were ordinated with t-distributed stochastic neighbor embedding (tSNE) using the R package Rtsne (Krijthe, 2015), and significance testing used many glm with negative binomial distribution using mvabund (Wang et al., 2012) because this approach takes into account heterogeneity in mean-variance relationships, i.e., autocorrelation (Warton et al., 2012). Differential abundance analysis was conducted using ANCOMBC with *P*-values adjusted by the Holm–Bonferroni method (Lin and Peddada, 2020). Calculation of geographic distances was performed using the "distGeo" function in the R package geosphere with WGS84 ellipsoid (Hijmans, 2019). Statistical modelling employed a general maximum entropy null model based on a bipartite configuration model for networks to model the location versus ASV occurrence matrix (Cimini et al., 2019). The *Z*-scores for nestedness were calculated using the NODF metric to indicate the number of standard deviations a given data point lay from the mean (Almeida-Neto et al., 2008). Estimation of niche overlap was achieved using the Jaccard distance to determine pairwise assemblage dissimilarity and test if the average dissimilarity deviated from that expected under random assembly that randomised ASV identity. Source tracking was conducted using fast expectation-maximization in the R package FEAST (Shenhav et al., 2019). R code used for FEAST is available at https://github.com/cyklee/STOTEN_FEAST.

3. Results and discussion

The sampling campaign encompassed twelve locations spanning the five major climate types and multiple biomes (Table 1). For all locations bacterial abundance per g soil greatly exceeded estimates per m^3 of air. Whilst such estimates between soil and atmosphere are not directly comparable the data illustrates that magnitude-scale differences in abundance occur and this pattern is conserved across multiple biomes on a global scale and broadly matches modelled estimates (Fig. 1a) (Burrows et al., 2009a). Taxonomic richness was also markedly higher in soils and the pattern for the ABL broadly tracked those for the location-specific soils (Fig. 1b). A clear trend in both abundance and richness was observed where more extreme surface biomes, e.g. Mauna Kea (high altitude mountain) and California and Chile (hot and cold deserts respectively) were associated with less abundance and richness in the overlying ABL.

Taxonomic richness of soil and ABL displayed a small but significant negative correlation with mean annual precipitation (MAP) ($R^2 = 0.06$, $P < 0.001$) (Fig. 2a). This pattern complemented a macroclimate-driven global latitudinal gradient observed for topsoil bacterial diversity (Bahram et al., 2018). The ABL taxonomic richness showed a small but significant positive correlation with mean annual temperature (MAT) ($R^2 = 0.1$, $P < 0.001$) that was not supported for soils (Fig. 2b). It is envisaged that locations with higher precipitation may support lower diversity in the ABL due to a variety of factors including precipitation-mediated deposition and less emissive surface soils. Conversely the increased diversity in warmer climates largely reflected more emissive soils (Maki et al., 2021; Salawu-Rotimi et al., 2021). However, our data showed that this relationship breaks down for the ABL above the most extreme desert locations, e.g. the Atacama Desert in Chile, and this likely reflects very low soil biomass at these locations.

We employed multiple approaches to unravel patterns in beta diversity and shed light on biogeographic patterns. Hellinger transformed Bray-Curtis distances were used to identify substantial dissimilarity between ABL communities (Fig. 3a). The habitat (i.e., ABL v. soil) and location were significant in structuring the composition of the bacterial assemblages (mvabun $P = 0.001$). The desert locations (Chile, Kuwait, Mongolia, Namibia) and the agricultural location (South Africa) displayed least within-group dissimilarity and this likely reflected less diverse surface sources for bacterial recruitment to the ABL. Soil communities followed a broadly similar trend where desert soils (Chile, Mongolia, Namibia) displayed lower within-group dissimilarity. We then estimated distance-decay relationships to estimate how community similarity varies with geographic distance that separates the communities. A significant though weak linear distance decay relationship for community structure was observed for both ABL ($R = 0.2175$, $P \leq 0.01$) and soil ($R = 0.147$, $P \leq 0.01$) (Fig. 3b).

To gain further insight we constructed a general maximum entropy null model of taxa occurrence matrices (Caruso et al., 2022), to provide statistical evidence for the degree to which communities in ABL and soil exhibited taxonomic structuring that would indicate non-random community structure. This is important because traditional dogma has long assumed microorganisms in the atmosphere are ubiquitously and randomly distributed. This identified that the atmospheric communities were significantly under-nested at the node level (Z score ≤ -2, $P \leq 0.001$) and the niche overlap (the degree by which taxa shared locations) was significantly lower (Z score ≤ -2, $P \leq 0.001$) compared to the null model (Fig. 3c). The two results indicated that ABL communities were taxonomically structured and fundamentally non-randomly assembled in terms of ASV identity. This is indicative of taxa specificity and reflects environmental filtering to habitat and location (Caruso et al., 2022). Soil communities displayed similar trends (Fig. 3c), although values indicated a greater degree of taxonomic structuring as expected for active soil bacterial communities and this is congruent with observed global diversity patterns for soil bacteria (Bahram et al., 2018). At the global scale ABL communities showed a higher level of similarity than soils due to the number of inter-location shared ASVs and greater location bias for soil (*Z*-score > 2, *P*-value < 0.001).

Assessment of taxonomic diversity in ABL and soil samples revealed the bacteria enriched in the ABL compared to soil were largely accounted for by classes with taxa that have known tolerance to environmental stress including Alphaproteobacteria, Bacilli, Cyanobacteriia, and Gammaproteobacteria (Fig. 4a). Other classes typified by stress-tolerant taxa, e.g., Deinococci, were also enriched albeit with low relative abundance. Other location-specific differences were indicative of influence from local surface cover (Fig. 4b). For example, the high relative abundance of Gammaproteobacteria in the farmland ABL in South Africa was consistent with emission signatures from agricultural surfaces (Salawu-Rotimi et al., 2021; Zhao et al., 2014), and the Cyanobacteria that are commonly elevated in marine aerosols were more abundant in the ABL at the coastal locations (Hawaii, Kuwait). The Mauna Kea location was a high elevation mountain peak (4200 m) and this gave some indication of likely atmospheric bacterial composition above the atmospheric boundary layer, with ultra-low biomass and diversity reflecting enrichment in stress-tolerant Cyanobacteria and Gammaproteobacteria.

A very conservative analysis of potential human-associated bacteria in the ABL was obtained by screening for twenty genera that contain known obligate human-associated species, although environmental species are also recorded for some of these genera and so this represents a cautious over-estimate: *Bacteroides*, *Bifidobacterium*, *Corynebacterium*, *Cutibacterium*, *Escherichia*, *Faecalibacterium*, *Haemophilus*, *Klebsiella*, *Lactobacillus*, *Listeria*, *Moraxella*, *Neisseria*, *Porphyromonas*, *Prevotella*, *Propionibacterium*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus* and *Veillonella*. The screening indicated that potential human contribution to environmental ABL communities was extremely low with a global average of 0.31 %. Urban aerosols were not the subject of our study but they may support elevated abundance of human-associated bacterial pathogens and allergens (Woo et al., 2013; Zhao et al., 2022). Human influence may also extend beyond the ABL with a recent study reporting detection of pathogenic bacteria in the free troposphere above the ABL (Triadó-Margarit et al., 2022).

Transit through the atmosphere is strongly related to survivability and the biocidal effects of high ultraviolet light, low temperature and low

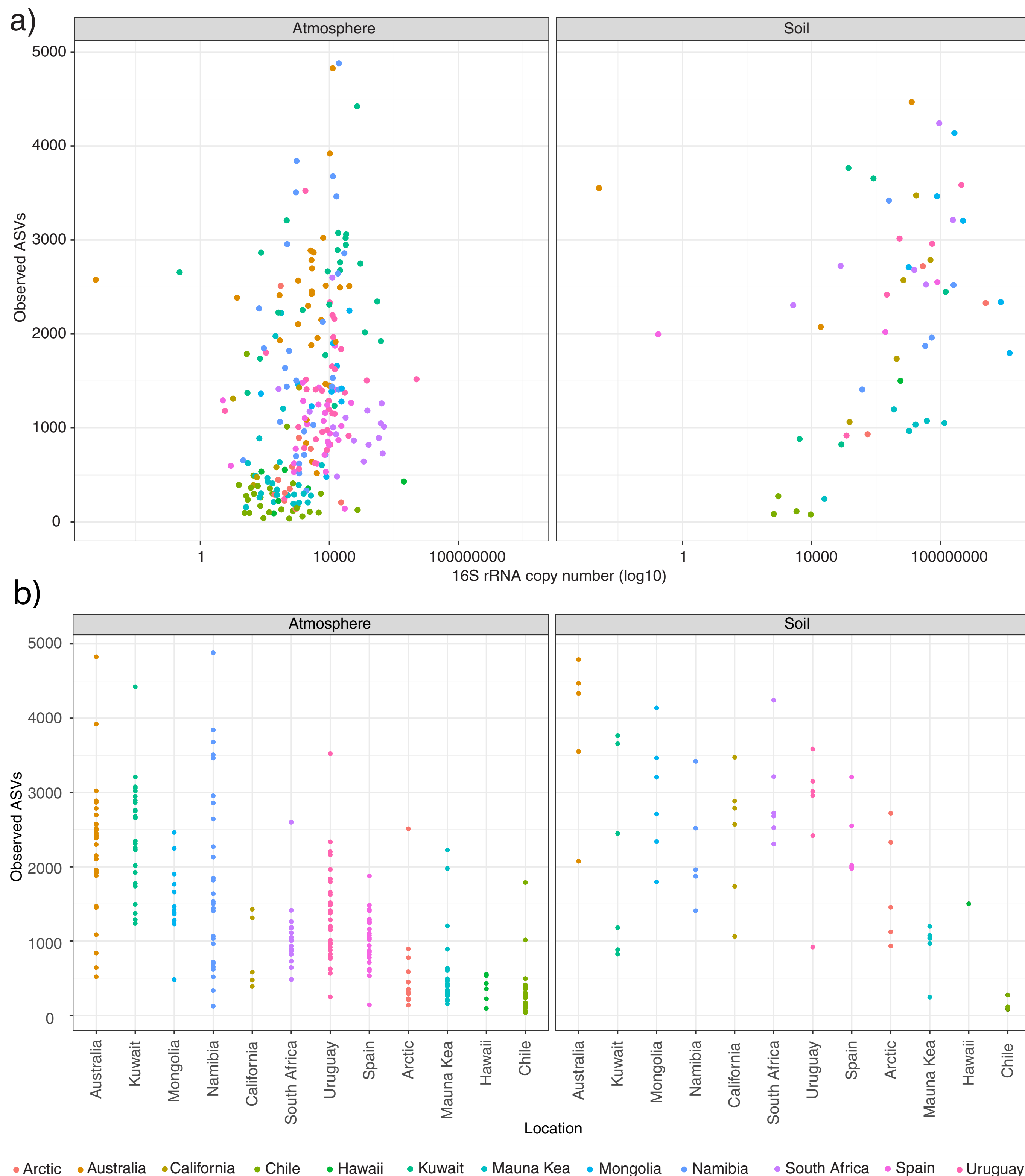

Fig. 1. Comparison of biomass and diversity for bacteria in the atmospheric boundary layer and underlying soil across biomes. a) Biomass-diversity relationship visualised using bacterial 16S rRNA gene copy number versus observed ASVs (standardised to: soil = g; atmosphere = m^3), samples with zero value for gene copy number estimation are not shown; b) Species richness of globally distributed ABL and soil bacterial communities, in rank order of richness in the ABL.

relative humidity have been identified (Brotto et al., 2015; Hara and Zhang, 2012; Prussin et al., 2017; Šantl-Temkiv et al., 2017; Tong and Lighthart, 2000). However, evidence also suggests that not all microorganisms become airborne in a uniform manner (Aalismail et al., 2019; Michaud et al., 2018), and some taxa may be adapted to enhanced deposition during transit (Reche et al., 2018). Our differential abundance analysis added statistical evidence in support of the taxa enriched in the ABL, and patterns also reflected potential differential adaptation to aerosolization and/or survival as well as source influences (Fig. 4c). For example, some classes such as the Actinobacteria and Gammaproteobacteria were common in both soil

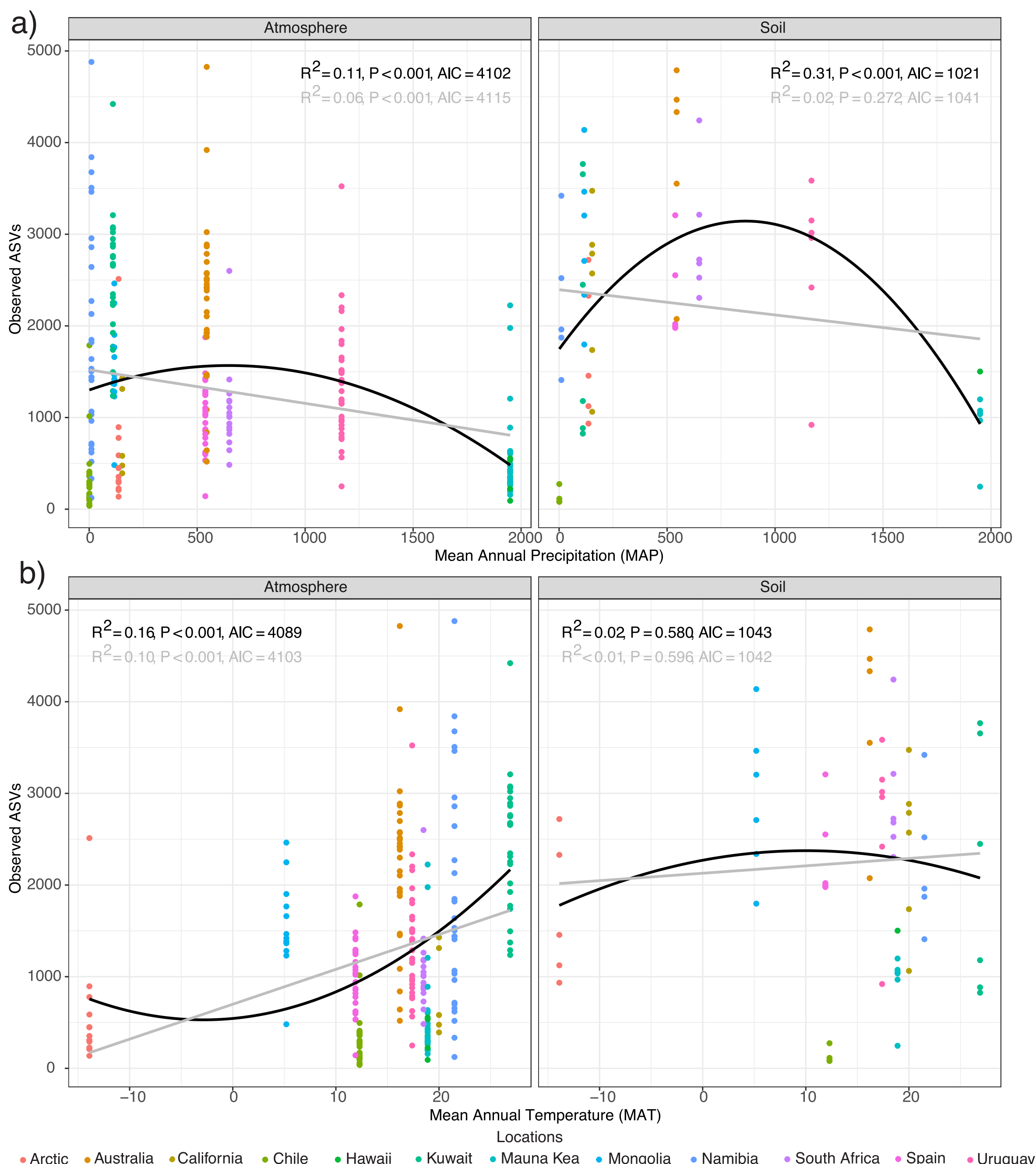

Fig. 2. The relationship between macroclimate and bacterial diversity in the atmospheric boundary layer and underlying soil across biomes. First and second order polynomial regression of ASVs by location for ABL and soil samples; a) The relationship with mean annual precipitation (MAP) was negative for ABL and soil communities; b) The relationship with mean annual temperature (MAT) was positive for ABL communities but not supported for soil communities.

and ABL communities, in contrast the Planctomycetes were consistently more abundant in soils, and this may indicate a group that is not well-adapted to aerosolization or atmospheric survival. Conversely the Cyanobacteria that are known to disperse readily in the atmosphere between habitats, e.g., (Pointing et al., 2009), and Deinococcota that are highly resilient to atmospheric abiotic stress (Satoh et al., 2022), were both more abundant in the ABL than soil.

A central question in atmospheric microbiology is where airborne taxa are recruited from, with the general presumption being that local sources predominate, particularly soils (Salawu-Rotimi et al., 2021), but also other terrestrial and aquatic surfaces, e.g. ocean surface waters (Mayol et al., 2017; Uetake et al., 2020), the phyllosphere (Lymperopoulou et al., 2016; Vorholt, 2012) and desert dust events (Kellogg and Griffin, 2006; Pointing and Belnap, 2012). A connectivity analysis of our data revealed that the ABL generally displayed greatest taxonomic connectivity with local soil at any given location and less connectivity with soil from different locations (two-way ANOVA with permutation test [5000 iterations] $P \leq 2.2 \times 10^{-16}$) (Fig. 5a). We then employed fast expectation-maximization source tracking (FEAST) (Shenhav et al., 2019), to estimate recruitment of ASVs to the ABL from soil samples collected concurrently at the same locations (Fig. 5b). The contributions of global soil ASVs to the ABL varied substantially with overall soil contribution ranging from 6.8 to 32.9 % of observed atmospheric bacterial diversity at any given location. This level of explained sources in our analysis is comparable to the 30 % achieved

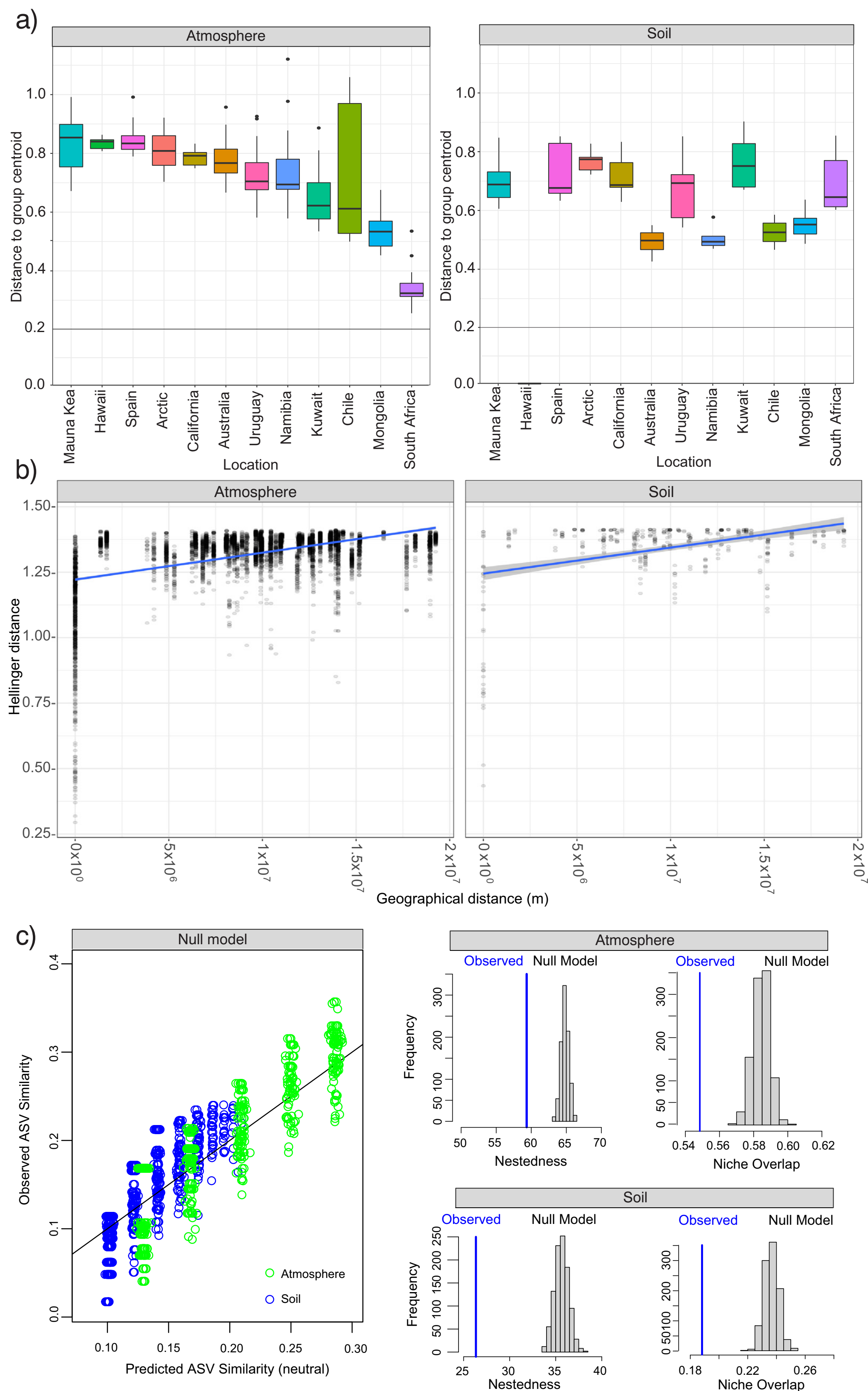

Fig. 3. Community assembly patterns for bacteria in the atmospheric boundary layer and underlying soil across biomes. a) Community dissimilarity visualised using Hellinger distance to centroid ranked my mean highest distance for ABL samples (low distance for Hawaii soil reflected fewer samples for this location); b) Bacteria in the ABL and underlying soil displayed significant though weak distance-decay relationships; c) Plot of predicted and observed ASV mean similarity against the null model (shown by the line), illustrating deviation of ABL and soil ASVs from the null model; subplots illustrate that bacterial communities in the ABL and soils were under-nested and therefore non-randomly assembled, and displayed reduced niche overlap compared with null models.

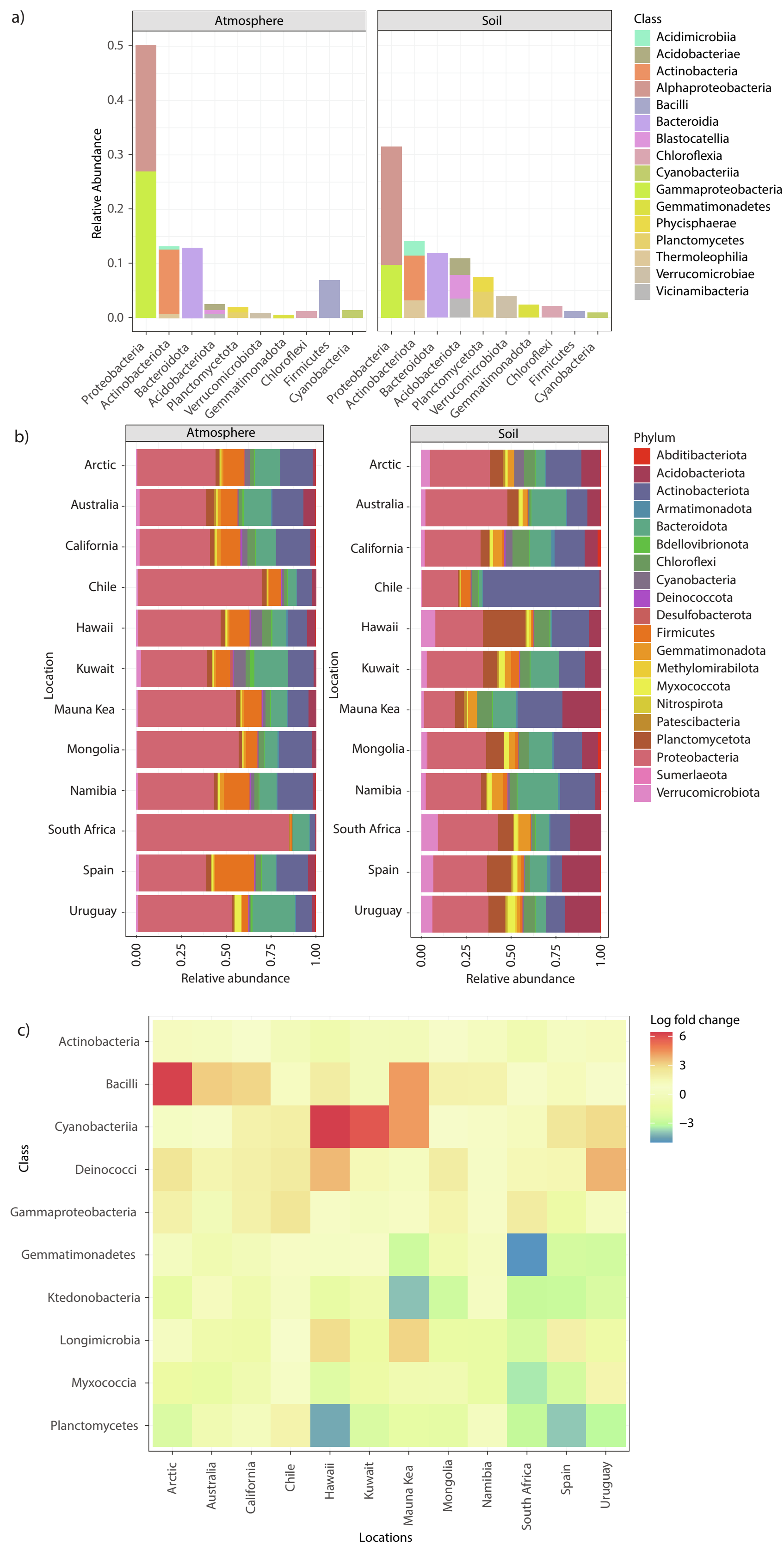

Fig. 4. Taxonomic composition for the atmospheric boundary layer and underlying soil across biomes. a) Global taxonomic composition of bacteria in the ABL and underlying soil, shown for phylum and classes >1 % mean relative, ranked my mean relative abundance in soils; b) Location-specific taxonomic composition of bacteria in the ABL and soil, shown for phyla >1 % mean relative abundance; c) Bias-corrected differential abundance analysis showing magnitude of differences (as natural log-fold change) for selected bacterial classes between the ABL and soil from the same location. Data are represented by a fold change ratio, where: >0 = more in the atmosphere, <0 = more in soil, 0 = no difference.

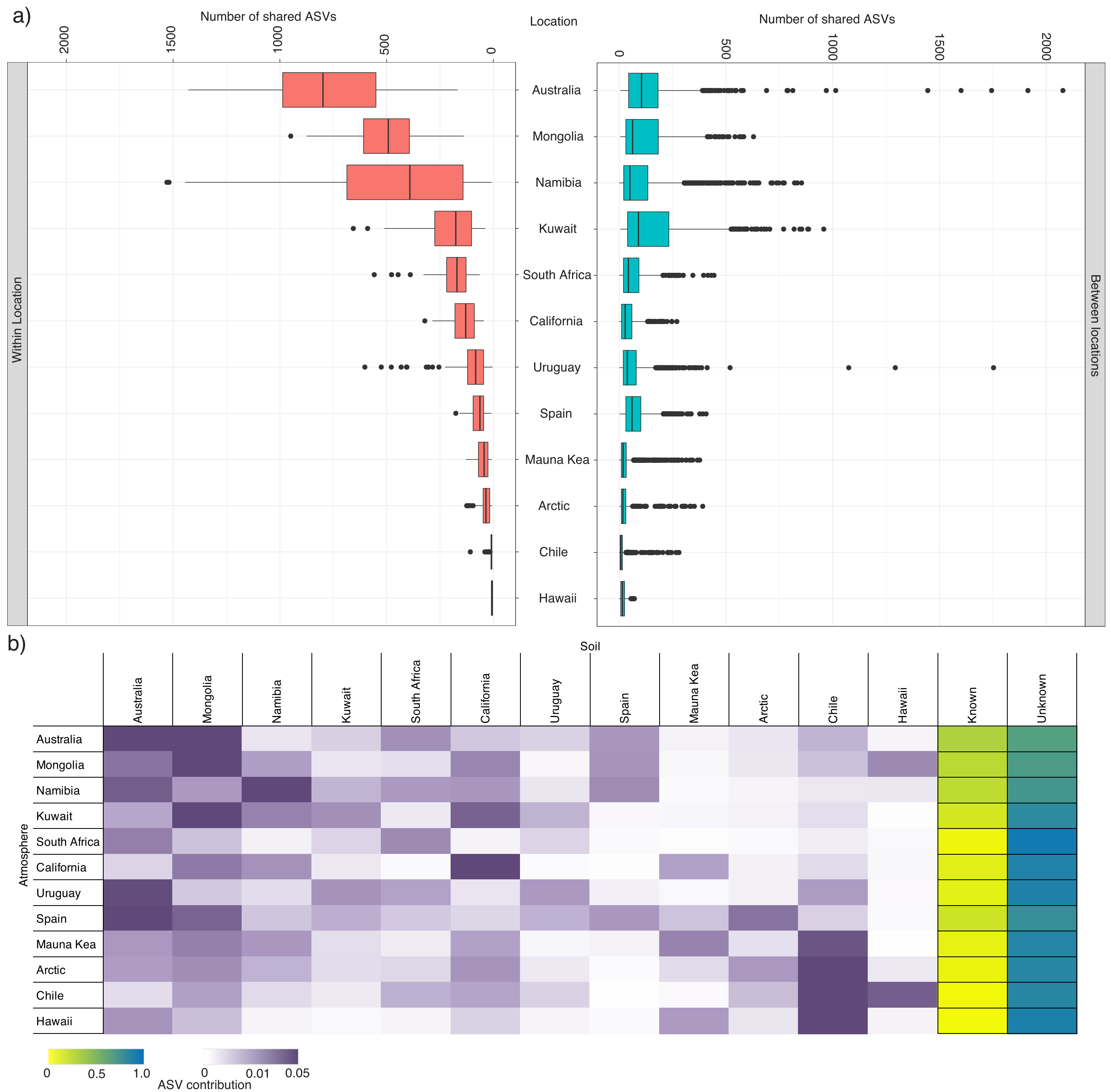

Fig. 5. Source tracking of bacteria in the atmospheric boundary layer from soil across different biomes. a) Comparison of the number of ASVs from the ABL recruited from soils from within and between different locations; b) Fast expectation-maximization source tracking of ASVs in the ABL that matched soil ASVs from multiple sources in this study. Data are represented as relative contributions using two colour scales: white-purple for sources recognised by data in this study, and yellow-blue for overall known and unknown sources.

in another recent study that employed FEAST source tracking to bacteria at the ASV level above the Southern Ocean (Malard et al., 2022). Another study under controlled conditions in a microcosm estimated that for bacterial taxa clustered at the genus level 19.5 % of airborne taxa could be explained by source tracking from a defined soil source whilst only 3 % were attributed to the phyllosphere source (Zhou et al., 2021). It is important to note that we matched exact ASV taxa in our FEAST source-tracking analysis and so our data revealed exact matches rather than the less specific approach of clustering operational taxonomic units (OTU) based on 97 % sequence similarity that has been previously applied to atmospheric source-tracking, and this resulted in a large volume of taxa with unexplained source and also highlights that it is impossible to exhaustively sample all potential sources.

The largest single contributor to the ABL identified from source tracking was derived from local soils, and this reinforced the findings from the connectivity analysis. Some locations also displayed relatively high non-local soil contributions. Environments with relatively stable, plant covered surfaces in the Arctic, Hawaii, South Africa, Spain, and Uruguay all had higher relative non-local soil contributors and generally had lower overall soil contributions than locations with more labile local surfaces in desert locations. This highlights both the importance of surface soil stability and the potential of phyllosphere-derived bacteria

to form a dominant part of the microbial community. Identification of diverse bacteria in aerosolised dust from agricultural and dryland soils has indicated the potential for soils to directly contribute to atmospheric microbiota (Elliott et al., 2019; Salawu-Rotimi et al., 2021). The direct emission of bacteria from phyllosphere to atmosphere has yet to be experimentally demonstrated but estimates derived from measurements of cultivable bacteria in air above grassland and forest suggest the phyllosphere is a major source (Burrows et al., 2009b).

4. Conclusion

Overall, the findings highlight that a clear cross-biome trend in diversity within the ABL occurs with regard to macroclimate variables, reflecting negative correlation with MAP and positive correlation with MAT. This generally matched observed trends for underlying soils although the relationships were more pronounced for ABL bacteria. Our statistical modelling confirmed that atmospheric bacterial composition was non-randomly structured and patterns reflected environmental filtering, thus refuting hypotheses that bacteria are ubiquitously distributed in the atmosphere, although at a global scale ABL bacterial communities displayed a higher level of similarity than soils. Soil was a significant source although other major inputs remain unexplained but are likely to include the phyllosphere as a major contributor. We conclude that diverse surface sources as well as temporal and stochastic variation in microbial occurrence within a given air mass likely all contribute to a complex ABL microbial community (Šantl-Temkiv et al., 2022). Taken together we anticipate these findings will be valuable in future hypothesis-driven research to identify interactions between the atmosphere and surface habitats across multiple ecological scales, and to test models of recruitment, turnover, functionality, and resilience. Given that the atmosphere is also the sink for a large fraction of anthropogenic emissions (Archer and Pointing, 2020), and climate change has been predicted to negatively impact microbial diversity in the ABL (Ontiveros et al., 2021), it is timely that this new contribution to the global inventory of microbial diversity is provided in order to expand a baseline for measuring future responses to change. Finally, the study complements efforts to inventory global environmental microbiomes (Thompson et al., 2017) and expands the scope of the pan-global microbiota.

CRediT authorship contribution statement

Stephen B. Pointing and Stephen D.J. Archer conceived and led the study; Stephen Archer performed laboratory experiments; Kevin C. Lee conducted bioinformatics; Tancredi Caruso performed ecological statistical analysis; Stephen B. Pointing, Stephen D.J. Archer, Kevin C. Lee, and Tancredi Caruso interpreted the findings; Stephen D.J. Archer, Kevin C. Lee, Antonio Alcami, Jonathan G. Araya, S. Craig Cary, Don A. Cowan, Claudia Etchebehere, Batdelger Gantsetseg, Benito Gomez-Silva, Sean Hartery, Ian Hogg, Mayada K. Kansour, Timothy Lawrence, Charles K. Lee, Patrick K.H. Lee, Matthias Leopold, Marcus H.Y. Leung, Teruya Maki, Christopher P. McKay, Dina M. Al Mailem, Jean-Baptiste Ramond, Alberto Rastrojo, Tina Šantl-Temkiv, Henry J. Sun, Xinzhao Tong, Bryan Vandenbrink, Kimberley A. Warren-Rhodes, and Stephen B. Pointing contributed to field planning and sampling; Stephen B. Pointing and Stephen D.J. Archer wrote the paper; All authors provided feedback and agreed the final manuscript.

Data availability

Data have been submitted to a publicly accessible database

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 data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162137.

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