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**Ecological succession of the microbial communities of an air conditioning
cooling coil in the tropics**

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Running Title: Microbial composition of an HVAC system

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Abstract

Air-conditioning systems harbor microorganisms, potentially spreading them to indoor environments. While air and surfaces in air conditioning systems are periodically sampled as potential sources of indoor microbes, little is known about the dynamics of cooling coil-associated communities and their effect on the downstream airflow. Here, we conducted a four-week time series sampling to characterize the succession of an air-conditioning duct and cooling coil after cleaning. Using an universal primer pair targeting hyper-variable regions of the 16S/18S Ribosomal RNA, we observed a community succession for the condensed water, with the most abundant airborne taxon Agaricomycetes fungi dominating the initial phase and *Sphingomonas* bacteria becoming the most prevalent taxa towards the end of the experiment. Duplicate air samples collected upstream and downstream of the coil suggest that the system does not act as ecological filter or source/sink for specific microbial taxa during the duration of the experiment.

Key words: Bacteria, Fungi, 16S, HVAC systems, airborne, cooling coil-associated microbial communities

Practical implications

In tropical environments, humans spend most of their time in building with air-conditioning systems. The air conditioning system can act as a population reservoirs or barriers to dispersal for microorganisms and thus affect the human health. Consequently, it is imperative to investigate the dynamic of the microbial communities on an air conditioning system.

Introduction

Airborne microorganisms can adversely affect human health through infections, allergic responses, and toxic effects (Spaces CoDI Health, 2004). In urban environments, residents spend on average 80% of their time indoors, where air is

48 treated by central heating, ventilation or air-conditioning (HVAC) systems (Klepeis et
49 al, 2001, Brasche and Bischof, 2005). Despite air conditioning systems being known
50 to harbor microorganism (Hugenholtz and Fuerst, 1992; Noris et al, 2011; Hospodsky
51 et al, 2012), the extent to which they can act as a source of microbes in indoor
52 environments and support microbial growth remains unclear. HVAC systems can
53 influence the structure and function of the indoor microbiome and thus impact human
54 health. This is of particular relevance in tropical environments where cooling coils in
55 centrally ventilated buildings are operated year-round, on a consistent and
56 continuous cycle during building operation hours.

57 In air conditioning systems, warm outdoor air passes over a refrigerant coil,
58 producing cooled air. During this process, warm air gets into contact with the cooling
59 coil and condenses on the coil surfaces, likely transferring microbial organisms on
60 the coil itself. The accumulation of condensed water generates a flux of dripping
61 water (Figure 1). In tropical climates, the coil surfaces experience water
62 condensation at each use whereas in temperate climate substantial period of
63 operation without condensation may occur. Condensed water can potentially
64 generate a habitat for microorganisms (Hugenholtz and Fuerst, 1992) and acting as
65 a source of airborne bacteria and fungi downstream. Previously, culture-based
66 methods have been used (Hugenholtz and Fuerst, 1992; Kemp et al, 2010) to
67 characterize the microbial composition of HVAC's components. Aside from the
68 limitations of culture-based methods (Rappé and Giovannoni, 2003), previous
69 studies were limited to capturing the microbial composition of HVAC's associated
70 components at a specific point in time, but did not provide insights on whether
71 communities change over time.

72 In this study we investigate the temporal dynamics in the taxonomic composition of
73 the microbial communities succession on an experimental cooling coil operated in a
74 tropical climate (outdoor-air), and their potential dispersal into downstream indoor air.
75 Using a cultured independent approach we characterize a microbial communities

associated with a cooling coil through a 4-week time series. In addition, we used both air and condensed water samples to investigate the impact of coil-associated microorganism on the air.

Methods

The HVAC system used for the experiment is a fin and tube cooling coil system situated between connecting upstream and downstream ducts. The fins and the duct are made of aluminum while the tubes are made of copper. The duct measures 4000 x 400 x 400 mm (LxWxH). It comprises an air-cooled condenser (Eden G4 Matrix EFPG series). The fan generates an air flow speed of ~1.5 m/s with the total flow rate around 1000 m³h⁻¹. Upstream air temperature was ~28°C, while the downstream was ~16°C. The rate of condensate water accumulation was approximately 0.5 mL/s. The system was detergent cleaned in all its parts including the ducting surfaces, condenser, heat disputing fins, and fan blades. The metal mesh covering the inlet of the coil had a pore size of 3.5 mm.

Sample collection

The HVAC system was run for a 9-h period between 9 am and 6 pm from Monday to Friday between Oct 27 to Nov 27 2014. The air and condensed water sampling were performed over the period of these four weeks. During the first three weeks, samples were collected from Monday to Friday, while for the last week samples were only collected Monday and Friday. All air samples were collected using a 0.22 µm pore-size polyester sulfonate (PES) membrane disk filter (Pall, Singapore) placed into a Sartorius Stainless steel in-line 47mm filter holders, connected to isokinetic sampling ports inside the duct. An ambient air sample was collected adjacent to the HVAC system intake at a height of ~1.5 m. Duplicate air samples were taken upstream and downstream of the cooling coil inside the aircon duct via a plastic tube connected to an isokinetic inlet inside the coil (Figure 1). The flow rate of the pumps was maintained at ~15 L/min for the entire 9-hr collection, as confirmed by measuring

the flow rate of each pump before and after sampling with a rotameter (SKC, Singapore).

Sterilization: Prior to sampling, the stainless steel filter holders were disassembled, soaked in 70% ethanol, rinsed in MilliQ water, soaked in 10% bleach solution, rinsed thoroughly with MilliQ water, and dried overnight in a 70°C oven. Wearing gloves and working inside a biosafety hood, flame-sterilized tweezers were used to place PES filters into each filter holder, which were then closed and the inlets and outlets sealed with parafilm. Filter holders were transported in a sealed container wiped with 70% ethanol to and from the sampling site.

Controls: Three different types of controls were included in this study. Handling controls were collected by placing a new sterile filter into a sterilized filter holder with flame-sterilized tweezers, closing the filter holder, then immediately transferring the filter to a 5 ml PowerWater® Bead tube. Passive sampling controls were collected by placing a filter holder with filter adjacent to the coil inlet for each sampling day, but not connected to any vacuum pump so no air was aspirated through it. Reagent controls consisted of new sterile filters placed directly into bead tubes. PCR was performed on the control DNA samples and no amplification was detected.

Collection: After sampling, the ends of the filter holders were sealed with parafilm and all filter holders were transported back to the laboratory for immediate transfer to 5 ml PowerWater® Bead tubes using flame-sterilized tweezers under a biosafety hood. All filter samples were stored at -20°C until further analysis.

Water samples: One litre of water condensing off the coil due to humid air passing over the low temperature refrigerant coils was collected at the start and the end of each day in autoclaved glass bottle via a plastic tube draining from the bottom of the coil (Figure 1). The water was immediately filtered through a 0.22 µm pore-size PES membrane disk filter and the filter was also placed directly into a 5 ml PowerWater® Bead tube and stored at -20°C until further analysis. Elemental analysis was

outsourced to SETSCO Services Pte Ltd, and performed using standard method APHA Method 3120.

Particulate matter counts

Counts of 0.3 – 10 µm particulate matter were recorded upstream and downstream of the coil systems using optical particle counters (OPC) (Model TSI Aerotrack 9306-V2 unit). Each OPC was connected to a plastic tube from an isokinetic inlet inside the coil, parallel to the inlets for the air filters. The flow rate was maintained at ~2.83 L/min and the particle cut point size for each channel was 0.3, 0.5, 1.0, 3.0 5.0 & 10.0 µm. Readings were collected each minute during coil operation.

DNA extraction, 16S PCR amplification and sequencing

The DNA extraction was performed using a modified protocol of the PowerWater® DNA isolation kit (MoBio Laboratories, Singapore) (Luhung et al, 2016). Briefly, an additional 30 minute incubation in a sonicating water bath at 65°C was added prior to beadbeating, and 10 µl (final concentration at 20 µg/mL) proteinase-K was added to each tube along with PW1. The final elution volume was also reduced to 60 µl, allowed to incubate the spin filter for 5 minutes at room temperature, and re-eluted through the spin filter.

DNA concentrations were determined with a Qubit® 2.0 fluorometer and a dsDNA High Sensitivity assay kit (Invitrogen, Singapore). Amplicon libraries were then generated using a modified version of the Illumina 16S Metagenomic Sequencing Library Preparation Protocol. Due to the low nucleic acids concentration for air samples, the number of cycles for the first PCR amplification was increased to 30. Universal primer pairs with an Illumina-specific overhang which target the V6-V8 hyper-variable region of the 16S/18S Ribosomal RNA gene were used (926wF_Illum:5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAACTYAAAKGAATTGRCGG and 1392R_Illum: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGGGCGGTGTGTRC) (Wilkins et al, 2013). Triplicate PCR reactions of each sample were pooled and

purified using Agencourt AMPure XP beads (Beckman Coulter, Singapore). Products from this first PCR step were then subjected to a second round of PCR to implement dual barcodes into each amplicon libraries, followed by another AMPure XP bead purification. Library quantitation was performed using Invitrogen's Picogreen assay and the amplicon library size was determined by running the libraries on a Bioanalyzer DNA 1000 chip (Agilent, Singapore). Library concentrations were normalized to 4nM and validated by qPCR on a ViiA-7 real-time thermocycler (Applied Biosystems), using qPCR primers recommended in Illumina's qPCR protocol and Illumina's PhiX control library as standard. Libraries were then pooled at equal volumes and sequenced in one Illumina MiSeq V3 sequencing run at a final concentration of 8.5pM and a read-length of 300bp paired-end with 15% PhiX control library spiked into the run. Data sets are accessible through NCBI's Sequence Read Archive (SRA), under study accession number SRP068997.

Sequences analysis

Reverse reads resulted to be of considerably lower quality as compared to forward reads: after quality trimming (threshold Q20), the big majority of reverse reads resulted to be cut to a length of ~100bp. This has made the paring of reverse and forward reads unfeasible. On the other hand, forward reads were affected by quality trimming only to a small extent, maintaining lengths close to 300bp. Because of that, it has been decided to perform the analysis using forward reads only.

After primer trimming, sequences were analyzed using the software QIIME (Caporaso et al., 2010). *De novo* OTU clustering at 97% similarity was performed using UCLUST (Edgar, 2010), representative sequences for each OTU were then blasted against SILVA database (Quast et al., 2013) version 111 (97% similarity). Samples that had less than 5000 sequences in the resulting OTU table were discarded from subsequent analysis. The samples that were discarded were the samples: C158, C069, C066, CW030. For the remaining samples, community

analysis was performed with normalized read depth equal to the number of sequences of the smallest sample (10665 sequences). No chimeric sequences were detected using ChimeraSlayer (List of QIIME commands can be found in the Supplementary Information).

Results and Discussion

An air-conditioning duct and cooling coil built in a laboratory environment was used to monitor microbial succession during operation (Figure 1). Before the start of the sampling period, the HVAC was disassembled, detergent-treated and dried in order to remove any previously accumulated matter. While this treatment did not sterilize the coil and duct, it was representative of commercial air conditioning systems cleaning. To simulate the operational features of commercial buildings, the system was run for an 9-h period (9am - 6 pm) over a span of 26 days, from Monday to Friday between Oct 27 and Nov 21 2014. Air and water samples were collected during operation as described in Figure 1 and the methods.

Air samples are dominated by Agaricomycetes

Using a universal primer pair targeting the V6-V8 hyper-variable regions of the 16S/18S Ribosomal RNA (Wilkins et al., 2013), the microbial community composition from air and condensed coil water were found to be significantly different (Supplementary Materials and Supplementary Figure 1). Air samples were dominated by Agaricomycetes which represented on average 67.1% of reads across all air samples respectively (Supplementary Figure 2).

Agaricomycetes mostly include outdoor mushrooms and polypores which are responsible for plant decomposition. Adams and colleagues (2013) also found Agaricomycetes as the most predominant fungal taxa in indoor environment during a mediterranean climate summer. This is also consistent with a previous study performed in urban areas from temperate regions, reporting that Agaricomycetes

were the most abundant group found within the Basidiomycota in outdoor air (Yamamoto et al., 2012). The Agaricomycetes, which are mushroom-forming fungi (Hibbett et al., 1997) generally do not include allergens or pathogens, with the exception of some Agaricales (Kern and Uecker, 1986). Using size-fractionation, Yamamoto et al (2012) also reported that fungi identified present in less than 4.7 μm size range were primarily Basidiomycota, with the majority of them in the 2.1-3.3 μm fraction (e.g. > 70-90%). This was likely due to the larger size of Ascomycete spores. Previous studies in the tropics using size-fractionated impaction reported that the majority of culturable fungi were found in sub-3.3 μm impactor ranges (Rajasekar and Balasubramanian, 2011; Balasubramanian et al., 2012). As our samplers did not have size selective inlets, Basidiomycota prevalence may be due to the effective size range sampled.

Previous culture-based studies in the tropics identified primarily Ascomycetes, with most common types including *Nigarsporia sphaerica* (*Khusia orzae*), *Cladosporium Curvularia* (Tan et al., 1992). On the other hand, previous studies in temperate regions found Dothideomycetes and Sordariomycetes as two most abundant fungal classes in airborne Ascomycota (Yamamoto et al., 2012; Fierer et al., 2008; Fröhlich-Nowoisky et al., 2009). Biogeographic patterns in outdoor air over continental scales have been driven by differences in temperature and humidity (Barberán et al., 2015). The difference in fungal composition is possibly a result of the tropical climate where our study was conducted whereas most previous studies were conducted in temperate regions (Barberán et al., 2014; Barberán et al., 2015). Indeed, a significant correlation of air microbial composition with humidity, precipitation and temperature was observed in our study (Supplementary Results). A global study of fungal composition of several geographically distributed indoor environments (Amend et al., 2010) had previously revealed that indoor environments in temperate zones show a greater diversity as compared to tropical climates, with the distance from equator being the best predictor of community similarity. This is consistent with our results of

the air samples being dominated by mainly one taxon (Agaricomycetes). Only a minor fraction of the air sequences were associated with Bacteria (19% on average), with *Perlucidibaca* (1.4%), *Acinetobacter* (1.4%) and *Enhydrobacter* (0.9%) (all belonging to the family of Moraxellaceae) followed by *Pseudomonas* (1.2%) being the most abundant ones. It is worthwhile to mention that a previous airborne metagenomic analysis from samples collected in two Singaporean shopping malls showed the air being dominated by the bacterial fraction instead, specifically with *Brevundimonas* as being the most abundant taxon (Tringe et al., 2008). Although the study was also conducted in Singapore, the nature of the indoor environment (characterized by a high human presence) does not allow for a direct comparison with our case. Our results also differ from a previous study looking at settle-dust microbial community in occupied university offices in temperate climates (Kembel et al. 2014). While *Deinococcus*, *Achromonobacter* and *Spiroplasma* were found to be the most abundant taxa in mechanically ventilated offices, *Methylobacterium*, *Sphingomonas* and *Streptococcus* were the most abundant in naturally ventilated offices.

Condensed water samples showed a community succession

Water samples also contained Agaricomycetes (Fungi), but at a lower abundance (18% on average across all water samples), and the bacteria *Sphingomonas* (18.1%), *Methylobacterium* (5.3%) and *Streptophyta* (6.2%). *Sphingomonas* and *Methylobacterium* are ubiquitous in the environment and were previously found to be easily isolated from air conditioning systems (Hugenholtz and Fuerst, 1992; Simmons et al., 1999; Schmidt et al., 2012). Their prevalence, together with the variation in Agaricomycetes abundance, were the main factors differentiating air from condensed water samples (SIMPER analysis).

The microbial taxonomic composition of the condensed water collected from the coil progressively changed over time, with the first and last day being the most distantly

related in terms of microbial composition. An early, middle and late phase have been identified as corresponding to days 1-5, 6-10, and 11-17 respectively (Figure 2A). In the early phase, condensed water samples had a high percentage of Agaricomycetes (24%) (Figure 2B), which progressively decreased over time (12% in the late phase), while *Sphingomonas* became the most abundant taxa in the water samples (5%, 21% and 30% in the early, middle and late phases respectively).

The diversity in condensed water samples was overall lower compared to air samples (Supplementary Figure 3) and was observed to progressively decrease over time (Supplementary Figure 4). This is consistent with the hypothesis of a rapid ecological succession following the initial settlement on the coil after the HVAC was restarted. In this respect, the coil would represent an empty habitat and the pioneer microorganisms of the coil by a metapopulation of Agaricomycetes would be a direct result of its high abundance in the environment rather than its fitness to life in the coil water.

Conversely, the presence of *Sphingomonas* later in the succession could be due to selection by copper in the external surface of the cooling coil tubes: *Sphingomonas spp.* have been found to contribute to the degradation of copper drinking water pipes (White et al., 1996). *Sphingomonas* can thrive under low nutrient conditions and are major players in biofilm formation (White et al., 1996). In addition, in the late phase a substantial presence of the fungal *Tremellomycetes* was detected (11%) while only negligible amounts were present in the early and middle phase. After 17 days of sampling (26 days from the beginning of the experiment) our results show that the community has not reached a stable state even if samples from the late phase are characterized by a smaller degree of dispersion (Figure 2A). Nevertheless this might represents a steady dynamic state of the system resulting from the continuous fluctuation of environmental parameters in tropical air.

We also hypothesize the existence of a “first flush” effect when the coil first turned on after sitting at high temperature and humidity overnight, and observed that total DNA yields were consistently higher in morning samples compared to evening samples (average DNA concentration morning, 0.45 ng/μl, evening 0.05 ng/μl) (Supplementary Figure 5).

This suggests that overnight, when the coil’s surface is not constantly flushed by the formation and dripping of condensed water there is an increase in biomass on the coil. However, although for some specific sampling days a great within day variation is observed (Figure 2A, days 1, 2, 7 and 9), the taxonomical composition of the microbial community did not differ significantly between the morning and evening samples (data not shown). This Indicates that the “first flush” effect does occur (biomass is flushed), but it has no detectable impact on the microbial community structure of the condensed water. Concentrations of dissolved iron, aluminum and copper were also measured for one of the samples, and resulted to be 2-7 times higher in morning water sample (Supplementary Table 1).

Interestingly, the sample collected on day 9 appears dominated by sequences classified as insects. Specifically, a large proportion of the insect associated OTUs can be identified as belonging to the specie *Pseudacteon obtusus*, a widespread fly used as a biological control for fire ants (Folgarait et al., 2005). Insects are known to harbor their own sets of microbes, which may influence the taxonomical classification obtained for day 9. Among the taxas detected in the sample of day 9, commonly associated with insects are *Pantoea*, *Klebsiella*, *Enterobacter*, *Acinetobacter*, *Pectobacterium*, *Sphingomonas*, *Pseudomonas* (Sabree et al., 2014; Engel et al., 2013). However, these taxas resulted equally abundant in samples from other days too, suggesting that the insect associated microbes did not impact the microbial composition of the sample up to an extent that was detectable under our experimental settings.

329

330 *The coil system does not act as a microbial source*

331 Air samples (upstream, downstream and ambient) clustered by day (Supplementary
332 Materials), highlighting the temporal variability of the microbial community
333 composition of the outdoor air. Ambient samples resulted to be significantly different
334 from upstream and downstream samples (Supplementary Results), such difference
335 can be observed in terms of alpha diversity as well (Supplementary Figure 3). This is
336 potentially explained by the 3.5 mm metal mesh covering the coil inlet (Figure 1 and
337 Supplementary Materials), which could impact particle deposition. In our system,
338 monitored for 26 days after being reverted to a pristine condition, the coil did not act
339 as a significant microbial source for downstream air. On average, for each sampling
340 day 0.91% of the sequences were detected downstream the coil and in the
341 condensed water, but not upstream. Analogously, 0.84% of the sequences detected
342 upstream, were found in the condensed water but not downstream. These
343 percentages are the result of variations in the presence/absence of multiple
344 OTUs/taxas, whose individual abundance is to be considered negligible or
345 indistinguishable from noise. OPC data used to monitor the abundance of different
346 airborne particles, did not highlight any removal or addition effect of the coil (data not
347 shown). No significant differences between upstream and downstream DNA yields
348 were found (Supplementary Figure 5). Our results diverge from another study
349 making use of the same coil system, where removal of airborne bacterial and fungal
350 DNA were observed (Wu et al, in review). However, in that case a HEPA filter had
351 been added to the rig and this likely resulted in removal of microbial populations.

352

353 The removal and addition of particulates from air-conditioning systems to the
354 downstream air has been shown using culture based approaches (Jo & Lee, 2008;
355 Kemp et al., 2010). In Jo and Lee (2008), the authors monitored bioaerosol
356 concentration inside a university seminar room, observing an increase in colony

forming units (CFU) following the activation of the HVAC system: elevated bacterial and fungal levels were only detected between 5 and 15 minutes after the system was turned on, with normal bioaerosol level gradually returning to baseline levels within 120 minutes, suggesting that the HVAC air flux disseminates significant amounts of viable organisms only for a short windows of time. Such shorts peaks in the bioaerosol levels would go undetected under our experimental settings, which require the air samplers to be operated for several hours for enough DNA to be collected.

Methodological considerations

Although this study showed an ecological succession of the microbial communities on an air conditioning cooling coil, there are some methodological limitations to be stated. First, as with most PCR-based studies, the choice of primers introduce potential bias. In this case, the 926F/1392R primers were used in order to evaluate both fungal and bacterial microorganisms. The relative abundance of fungi is probably overestimated, given that the high numbers of rRNA gene copies in fungal (Maicas et al. 2000; Tsuchiya and Taga, 2001) and thus make the detection of fungal in our samples more sensitive. Lastly, the time lapse used to investigate the coil succession might be short. However, previous studies has shown that biofilm does form over this time period on the same scale that we measured (Schmidt et al., 2012).

Conclusion

This study presented the temporal dynamics of the microbial communities on an experimental cooling coil operated in a tropical climate, and their potential dispersal into downstream indoor air. The samples collected upstream and downstream of the coil suggest that the system does not act as ecological filter or source/sink for specific microbial taxa. However, additional factors including operation over longer periods or at different flow rates could affect whether coils installed in typical HVAC

systems might act as population reservoirs or barriers to dispersal for specific microbial taxa.

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Figure 1: HVAC schematic representation with sampling setup: For each sampling day, duplicate air samples were collected upstream and downstream of the cooling coil, together with a single ambient air sample placed adjacent to the coil intake. Water condensing off the coil was collected at the beginning (just after turning the system on) and at the end (just before turning the system off) of each sampling day.

Figure 2

Panel A: Evolution of the microbial community succession on the coil surface.

MDS plot of the Bray-Curtis dissimilarity between different water samples,. For each

day, AM and PM samples were merged. **Panel B: Taxonomic composition of**

water samples. Percentage of abundance of the most abundant OTUs is shown by

days (x axis). For each day, AM and PM samples were merged. Numbers on x-axis

indicates the day.

Supplementary Information

Ecological succession of the microbial communities of an air conditioning cooling coil in the tropics

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Running Title: Microbial composition of an HVAC system

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Supplementary Results

Statistical analysis

Statistical tests were performed using permutational multivariate analyses of variance (1) with the PERMANOVA tool in PRIMER (2). Bray-Curtis similarity matrices of square root-transformed relative abundances were generated. P-values were calculated on 9999 permutation under a reduced model. Air samples were significantly different from water samples ($P=0.0002$). For subsequent analyses air and water samples were treated separately.

Air samples: The factor 'location' was fixed with 3 levels (upstream/downstream/ambient) and factor 'day' was random with 17 levels. A significant effect of both 'location' and 'day' was detected ($P=0.0001$). Pair-wise test on the factor 'location' highlighted a significant difference between 'ambient' and 'downstream', and between 'ambient' and 'upstream' ($P=0.0001$ in both cases). No significant differences were detected between 'upstream' and 'downstream' ($P=0.1721$). Relationships between air samples composition and a set of environmental parameters were analyzed using a distance based linear model (DISTLM) (Anderson *et al.*, 2007), using the BEST selection procedure and the AIC selection criteria. Both the average relative humidity and the total amount of rain recorded during the coil operational time resulted to a significant contribution to the variation in microbial composition of air samples (marginal tests $P=0.0001$ and $P=0.0007$ respectively). Same conclusion apply to the average temperature, which has been excluded from the test due to its high correlation with the average relative humidity.

Water samples: The factor 'phase' was fixed with 3 levels (early/middle/late), factor 'day' was random, nested in 'phase', with 17 levels. A significant effect of 'phase' was detected ($P=0.0001$). Subsequent pair-wise test on the factor 'phase' showed a significant difference between 'early' and 'middle' ($P=0.0074$), 'middle' and 'late' ($P=0.0076$), and 'early' and 'late' ($P=0.0049$).

QIIME Commands

```
REP_SET=/Path_to_Silva/rep_set/97_Silva_111_rep_set.fasta
REP_SET_ALIGN=/Path_to_Silva/rep_set_aligned/97_Silva_111_rep_set.fasta
TAXA=/Path_to_Silva/taxonomy/97_Silva_111_taxa_map.txt

split_libraries_fastq.py [full command omitted due to high number of samples]

pick_otus.py -i seqs.fna -o seqs_otus.txt

pick_rep_set.py -i seqs_otus.txt -f seqs.fna -o reps_set.fna

parallel_assign_taxonomy_blast.py -i rep_set.fna -r $REP_SET -t $TAXA -o
taxonomy_assignments

parallel_align_seqs_pynast.py -i rep_set.fna -o alignment --template_fp
$REP_SET_ALIGN

identify_chimeric_seqs.py -m ChimeraSlayer -i rep_set_aligned.fasta -a
$REP_SET_ALIGN -o chimeric_seqs.txt

make_otu_table.py -i seqs_otus.txt -f
taxonomy_assignments/rep_set_tax_assignment.txt -o otu_table.biom

biom summarize-table -i otu_table.biom -o otu_table_summary.txt

#useful to run here for decision on sampling depth to use for normalization
alpha_rarefaction.py -i otu_table.biom -m mapping.txt -o parameters.txt -o
rarefaction

single_rarefaction.py -i otu_table -o otu_table_even.biom -d 10665

summarize_taxa_through_plots -i otu_table_even.biom -o taxonomy_assignments -
m mapping.txt

alpha_diversity -i otu_table_even.biom -o alpha_diversity -m observed_species
```

References

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Supplementary Table 1: Element analysis of morning (AM) and evening (PM) water samples.

Sample	Copper as Cu (mg/L)	Aluminum (mg/L)	Iron as Fe (mg/L)
Water AM (sampling day 15)	0.020	0.20	0.90
Water PM (Sampling day 15)	0.0075	0.073	0.12

Supplementary Figure 1: MDS plot of Bray-Curtis dissimilarity between air and water samples. Sampling day is shown as label.

Supplementary Figure 2: Taxonomic composition of air samples. Percentage abundance of the 15 most abundant OTUs is shown by sampling day. For each day, upstream, downstream and ambient samples are shown; for upstream and downstream, the average of the duplicates was used. Data was square-root transformed to highlight the contribution of less abundant species.

Supplementary Figure 3: Boxplot representation of alpha diversity (number of OTUs) for upstream air, downstream air, ambient air and condensed water samples. Confidence interval of median 95%.

Supplementary Figure 4. Rarefaction curves based on number of observed taxa with respect to early, middle and late phases.

Supplementary Figure 5: Boxplot representation of upstream and downstream DNA yields of air samples. Confidence interval of median 95%.