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Characterisation of fungal and bacterial dynamics in an active green wall used for indoor air pollutant removal

Fleck. R [a]*, Gill. R.L. [ab], Pettit. T [a], Irga. P.J [d], Williams. N [c], Seymour. J [c], Torpy. F.R. [a].

[a] Plants and Environmental Quality Research Group, School of Life Sciences, Faculty of Science, University of Technology Sydney, Australia
[b] Coastal Oceanography and Algal Research Team, Faculty of Science, Climate Change Cluster, University of Technology Sydney, Australia
[c] Ocean Microbes and Healthy Oceans, Faculty of Science, Climate Change Cluster, University of Technology Sydney, Australia
[d] Plants and Environmental Quality Research Group, School of Civil and Environmental Engineering, Faculty of Engineering and Information Technology, University of Technology Sydney, Australia

*Corresponding Author. University of Technology Sydney, P.O. Box 123, Broadway, NSW 2007, Australia. *E-mail address:* Robert.Fleck@uts.edu.au (R. Fleck).

Abstract

Indoor air quality (IAQ) is of growing public health concern which has prompted the use of plants to phytoremediate air pollution in interior spaces. Active green walls are emerging as a means of reducing indoor contaminants and have demonstrated efficacy comparable to conventional air filtering technologies. However, the use of active airflow through organic substrates has the potential to emit bioaerosols into the surrounding environment, where the potential risk to human health is largely unknown. In this study, we demonstrate that two indoor green walls (with and without active airflow) contribute significantly to the ambient fungal load, however concentrations remained well below WHO safety guidelines. Bacterial dynamics within the rhizosphere/substrate of the operational botanical biofilters displayed variability across plant species. Phyla-wide distribution generally aligned with previous literature; however, differences from those previously reported were observed at the genus level, possibly due to geographic location, substrate composition, or plant species selection. Targeted assessment of *Legionella* aerosol contamination, an under-addressed potential pathogen for these active systems, yielded no positive identification during the sampling period. We conclude that active green walls host a unique bacterial profile and do not emit harmful levels of fungal propagules or pose significant risk of aerosolised *Legionella* species, provided systems are well monitored and maintained.

Keywords

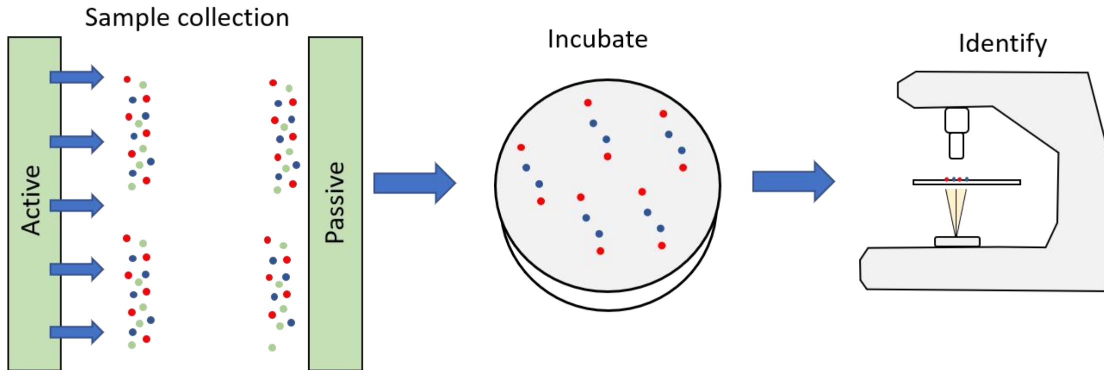
Indoor air quality (IAQ), Active green walls, Botanical biofilter, Bioaerosols, Bacterial characterisation, Legionella.

Highlights

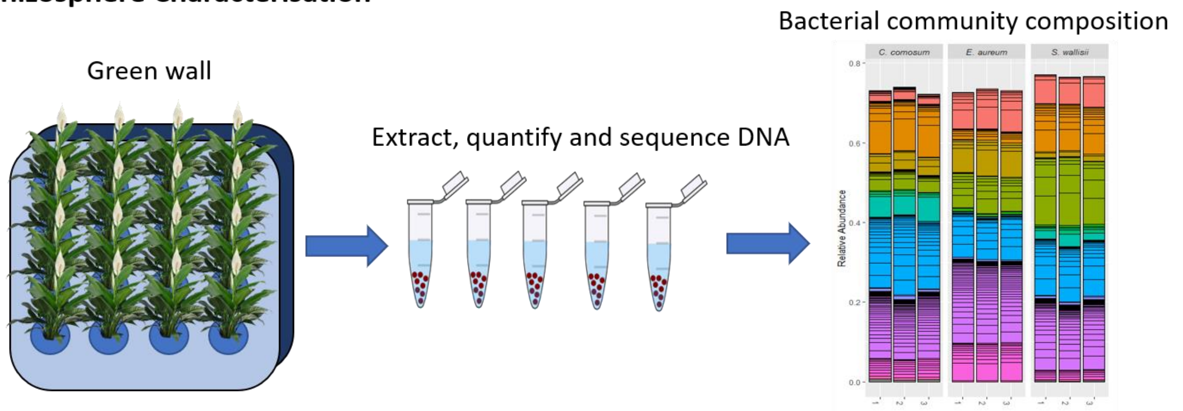
- Green wall bacterial community varied amongst plant species.
- The aerosolised fungal load emitted by green walls did not exceed WHO guidelines.
- No aerosolised *Legionella* species were identified.

Graphical Abstract

Bioaerosol Assessment



Rhizosphere Characterisation



44 ## 1. Introduction

45 In modern societies, humans spend up to 80% of their time indoors [1], where air quality is often more
46 polluted than outdoors [2,3]. Due to the accumulation of air pollutants, and the extended duration of
47 exposure associated with an indoor lifestyle [4], domestic and commercial indoor air pollution is responsible
48 for up to 5% of the global disease burden [5], equating to costs of approximately US$90 billion annually [6].

49 Since the 1980s, the use of plants in interior spaces to phytoremediate air pollution has grown considerably
50 in popularity [7,8]. The efficiency of botanical systems in improving indoor air quality has been significantly
51 enhanced by the development of active botanical biofiltration, or active green wall systems [9]. Active green
52 walls use ornamental plants grown along a vertical plane with the addition of mechanical air induction to
53 actively draw polluted air through the plant growth substrate and foliage [10]. During this process, air
54 pollutants are delivered directly to the rhizosphere where they may be metabolised/sequestered by
55 microbes, the predominant mechanism for contaminant degradation [11–13]. Additionally, particulate
56 matter (PM) may be filtered by the substrate and root structures [14].

57 While botanical biofiltration is still an emerging technology, there is substantial evidence for its practical
58 potential, along with growing commercial interest [15–19]. In their current state, botanical biofilters have
59 comparable removal efficiencies to those of conventional indoor technologies such as MERV (minimum
60 efficiency reporting value) 4, 6, 10, 11 and 13 filters for the removal of PM (PM_{10} and $PM_{2.5}$) [20]. In addition,
61 botanical biofilters are capable of reducing indoor concentrations of volatile organic compounds (VOCs) and
62 other pollutants such as CO and CO_2 [21–25], which cannot be removed by most conventional systems, other
63 than by dilution [26].

64 Despite the benefits of active green wall technologies, there is a potential for systems that use active airflow
65 through biologically active substrates to emit bioaerosols into the surrounding environment [27]. It has
66 indeed been proposed that active green walls may provide a favourable environment for the proliferation of
67 pathogenic fungal or bacterial species, with the use of mechanically assisted air flow increasing the risk of
68 the aerosolisation of water containing microbial bioaerosols. Currently, research which has assessed
69 bioaerosol emissions from active green walls are limited to assessments of total fungal and bacterial loading.
70 While there are no documented cases where harmful levels of fungal [28–31] or bacterial aerosols
71 [20,28,31,32] have been detected in active green wall emissions, there is a paucity of research that has
72 comprehensively characterised bioaerosol emissions, and we propose that assessments of this kind are
73 essential to fully understand the implications of biowall systems for indoor air quality (IAQ).

74 Limited research has specifically investigated the aerosolised release of pathogenic bacteria from green walls
75 [33], such as the ubiquitous bacterial genus *Legionella*. *Legionella* are free-living motile bacteria that can
76 infect other microorganisms or form chemo-resistant biofilms [34–36], and several species are the causative
77 agents of legionellosis [36–38]. *L. pneumophila* serogroup 1 is responsible for up to 90% of infections
78 worldwide, with the exception of in Australia, New Zealand and Thailand, where *L. longbeachae* is the
79 dominant pathogen, and is responsible for up to 40% of infection [39–42]. The dispersal mechanisms of these
80 two species vary significantly [33,43]. Where *L. pneumophila* requires aerosolization through water droplets
81 for infection to occur [33,37,38,44], *L. longbeachae* requires physical contact from contaminated soils with
82 the eyes or mouth [42,43]. Due to the nature of the components used in active green walls, there is some
83 concern that *Legionella* spp. may proliferate within irrigation systems and botanical substrates and become
84 aerosolised in the event of over-watering or physical disturbance.

85 In this study, we aimed to determine whether an established active green wall in a modern urban office
86 building contributed significantly to the release of fungal and bacterial aerosols, with specific focus on
87 bioaerosols that have implications for IAQ and human health. We assessed the culturable indoor
88 aeromycota, characterised the bacterial community composition using 16S rRNA amplicon sequencing
89 approaches, and performed targeted enumeration of the pathogen *Legionella* spp. to examine potential risks
90 to public health.

2. Methodology

2.1 Site description

Aerosol sampling was conducted on four floors (levels 12 – 14 and 17) of a newly built commercial office building, made of steel and glass near Sydney's Central Business District. The building featured standard heating, ventilation and air conditioning (HVAC) systems with no additional filtration technology. One active and one passive green wall span the interior of two stories (levels 13 and 14), each covering 60 m^2, in a semi-open plan café and meeting/reception space, with a floor space of 2300 m^2. Both green walls consist of several hundred individual plant housings (modules) supporting sixteen plants per module [45]. These house six plant species: *Chlorophytum comosum, Spathiphyllum wallisii, Epipremnum aureum, Gibasis* sp.*, Philodendron xanadu* and *Peperomia obtusifolia*, of which *C. comosum, S. wallisii, E. aureum* represent most of the greenery at the time of sampling.

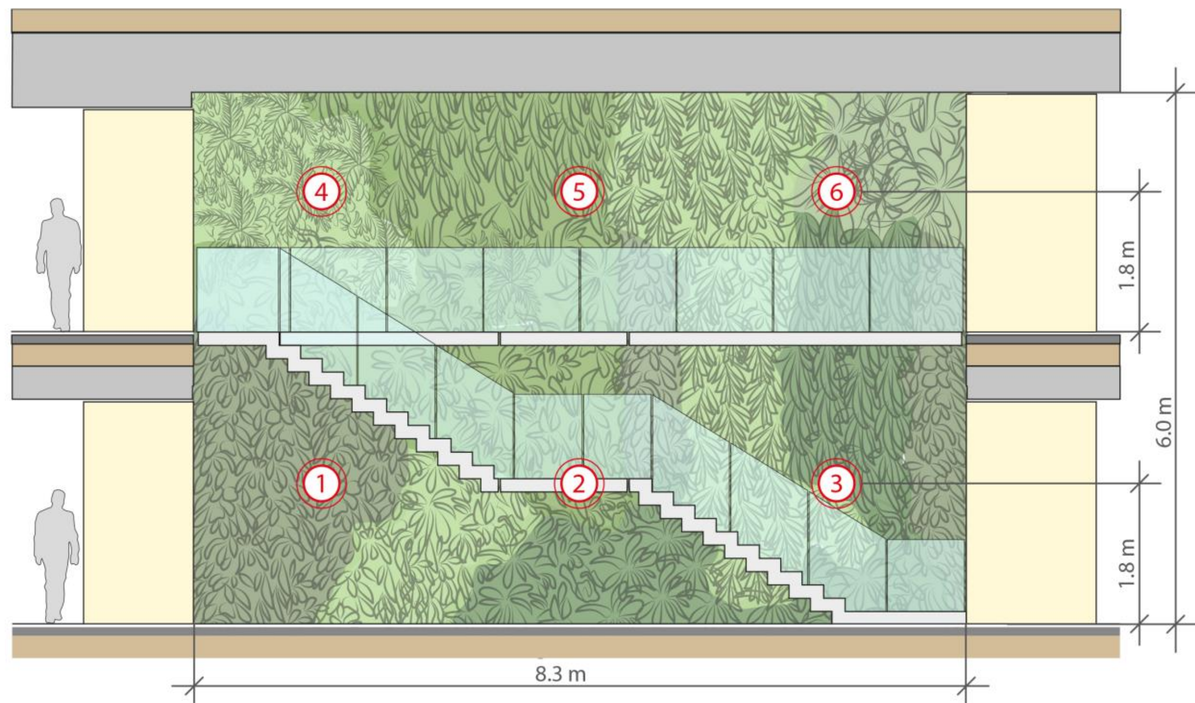
The active green wall utilises six low-profile 230 V, single-phase, 50 Hz fans operating at 98.96 m^3/h, per fan, when freestanding. These are located at the bottom of the wall to facilitate active air flow through the plant growth substrate and foliage. Each fan supplies airflow to 9.5 m^2 of active green wall on 15-minute on–off intervals, from 06:00 to 20:00 daily, with a total run time of seven hours per day. The volumetric flow rate of effluent air was recorded as 17.3 m^3/h through the green wall, determined with a VELOCICALC Model 9545-A air velocity meter (TSI Incorporated, USA). Active and passive walls are irrigated every two and four days respectively for eight minutes (two 4-minute watering cycles), with a delivery rate of 5 L/minute. Irrigation is divided into zones, with three irrigation catchments. Runoff falls gravimetrically into large drainage reservoirs at the base of the wall.

To determine the bioparticle density in areas of the building not serviced by the green walls, reference sites were positioned on levels 12 and 17 in thoroughfares within the building, as the open plan office spaces of these floors contained relatively high densities of potted plants (~0.33 potted plants/m^2) which may have also had an effect on indoor bioaerosol levels [13].

Foot traffic was explored by correlation analyses (Pearson's correlation coefficient) to assess its association as a potential influential variable with fungal density [46]. Foot traffic data was sourced from the building reception booking system.

2.2 Bioaerosol assessment

Bioaerosols were sampled over a three-month period from late summer to early autumn (February 28th, March 28th and May 3rd, 2019) between 11:00 and 14:00. Samples were collected with a Reuter Centrifugal Sampler (RCS; Biotest Diagnostics Corp., Denville, NJ, USA), which is comprised of a hand-held cylinder with an impeller that rotates at 4100 rpm. Air is drawn into the sampling head, and aerosolised particles are imbedded on the surface of selective agar strips that encircle the head. The RCS was operated for 2- and 4-minute cycles, sampling 80 and 160 L of air for fungal and bacterial samples respectively. For each green wall, six samples were taken at different locations adjacent to the wall surfaces. On the lower level of each green wall, three samples were taken along the length of the wall 1.8 m above ground and 0.5 m from wall surfaces, clear of any protruding vegetation. This sampling procedure was repeated on the upper level of each green wall. Thus, the sampling locations encompassed the length and height of the green walls, as well as taking air samples from places that were relevant to a person's breathing zone. Reference site samples (six in total) were collected from designated 3 m^2 spaces within the thoroughfares, 1.8 m above ground and 0.5 m from wall surfaces. Sample collection was conducted at each location individually, and all samples were completed within a three-hour window and during the operational period of the active green wall fans.



136

137 Figure 1: Schematic representation of bioaerosol sampling locations across each green wall site of a newly
138 built commercial office building. Sampling was conducted over two stories, 1.8 m above ground and 0.5 m
139 from protruding vegetation at both sites.

140

141 Commercial Rose-Bengal Chloramphenicol (RBC) agar RCS strips and modified RCS strips containing Buffered
142 Charcoal Yeast Extract with L-Cysteine agar (BCYE-cys) were utilised for fungal and *Legionella* sampling
143 respectively. RBC is a pH neutral agar with added Chloramphenicol for the suppression of bacterial growth
144 and allows control of the size and height of mould colonies to prevent luxuriant species growing over slow-
145 growing moulds or yeasts [47]. Bacterial samples were assessed for L*egionella* spp. as per the British
146 Standards Institution (BSI) [48]. Incubated BCYE-Cys agar strips were assessed visually for putative *Legionella*
147 colonies (grey/white in colour) and, if detected, colonies were sub-cultured onto BCYE growth agar, deprived
148 of L-cysteine, where *Legionella* spp. should not grow. BCYE-Cys is a *Legionella* spp. preferential growth media
149 that employs the use of L-Cysteine, soluble ferric pyrophosphate and alpha-ketoglutarate to enhance
150 *Legionella* growth. The use of activated charcoal removes toxic metabolic products and proteins, and other
151 growth nutrients are supplied by yeast extract [49]. All samples were tested against a *Legionella pneumophila*
152 positive control.

153 Imbedded fungal and bacterial samples were sealed and transported for incubation at room temperature
154 (21–23 °C) and 37 °C respectively in dark, aerobic conditions for five days. Cultured strips were photographed,
155 after which colonies were enumerated. Fungal samples were identified by microscopy using identification
156 guides [50–52]. Colonies that did not have conidial structures or spores were classified as 'sterile mycelia'.

157

158 ***2.3 Characterisation of bacterial community diversity***

159 Samples from three single-species (*C. comosum, E. aureum* and *S. wallisii*) botanical biofilter modules
160 containing healthy plants were selected randomly from the active green wall. Modules were suspended and
161 flushed with 12 L of Milli-Q water (Ω 18.2; Millipore, Eschborn, Germany) and run-off was collected in pre-
162 sterilised (1:10 sodium hypochlorite, Milli-Q rinsed) natural LDPE plastic bags. Samples were then aseptically
163 transferred to three sodium hypochlorite sterilised 10 L sample containers for filtration. Triplicate 2 L samples

164 were filtered through 0.22 µm GPS sterivex membrane filters (Millipore). Filters were stored at -80 °C prior
165 to DNA extraction.

166 DNA extraction was performed using the DNeasy PowerWater kit (QUIAGEN) as per the manufacturer's
167 instructions. A Nanodrop-1000 spectrophotometer was used to measure DNA quantity and purity. DNA was
168 sequenced using the 16S rRNA amplicon Illumina MiSeq platform (2 x 300 bp), by the Ramaciotti Centre for
169 Genomics (University of New South Wales, Sydney, Australia), using the V3-V4 region (341f-805r) and 341F
170 and 805R primers.

171 16S rRNA fastq files were processed using R-Project and the Dada2 (V1.12.1), DECIPHER (V2.12.0), and
172 Biostrings (V2.52.0) packages [53–55]. High quality reads were filtered and trimmed using: trimLeft = c
173 (17,21) to remove primer sequence; truncLen = c (280,210) to trim low-quality tails; and maxEE (2,5) to relax
174 the expected error on the reverse sequence. Taxonomic classification was assigned by aligning amplicon
175 sequence variants (ASVs; equivalent to operational taxonomic units (OTU) at 100% sequence identity) with
176 the Silva SSU r132 database [56]. A grouped mean relative abundance (gmRA) threshold of ≥100 was
177 established as a cut-off for plotting using the phyloseq (V1.28.0) and ggplot2 (V3.2.0) packages [57,58].

178

179 ### ***2.4 Statistical analysis***

180 Fungal density data was rank transformed prior to analysis to improve homogeneity of variance across
181 treatments. A rank transformation was chosen over a logarithmic function due to the nature of the error
182 distribution in all samples, where variance was highly skewed above the mean, relative to below it. A two-
183 way repeated measures analysis of covariance (2-way RM ANCOVA) was performed to assess differences in
184 rank-transformed fungal bioparticle density (CFU/m^3) between green wall sites and through time, with foot
185 traffic (number of passers-by per sampling period) as a covariate (Pearson's $r = 0.651$). Tukey's Honest
186 Significant Difference (Tukey's HSD) was performed on significant effects to evaluate comparisons. Further,
187 Shannon's H-Index was calculated to assess fungal diversity between green wall sites and across time points,
188 and a two-way RM ANOVA was performed to analyse differences. Results were deemed significant at $\alpha =$
189 0.05. All analyses were performed in R-Project, version 3.6.1 [59].

190 To assess differences in bacterial community diversity, a non-metric multidimensional scaling (nMDS) biplot
191 was generated (Supplementary 1) using relative abundance taxa data. Observed differences amongst
192 botanical modules with different plant species were then examined with permutational analysis of variance
193 (PERMANOVA), and the contribution of individual phyla to dissimilarities amongst plant species was assessed
194 with analysis of similarity percentages (SIMPER, Bray-Curtis similarity index). These analyses were performed
195 with the Vegan (V2.5.5) package [60] in R-Project.

196

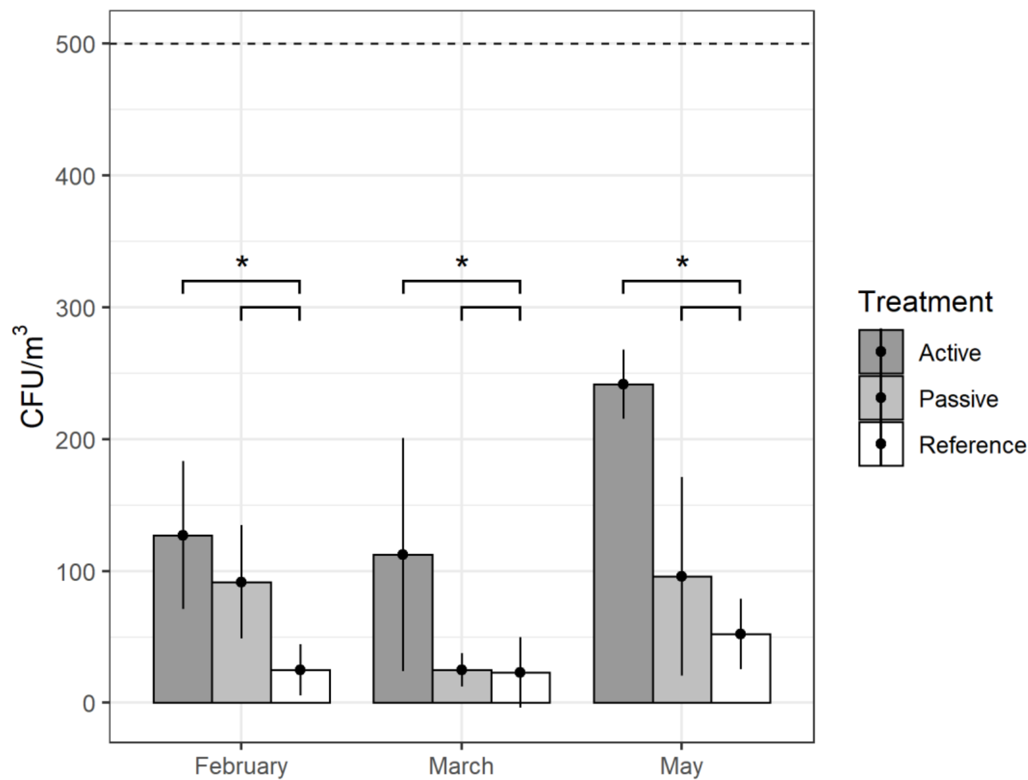
197 ## 3. Results:

198

199 ### ***3.1 Fungal bioaerosol assessment***

200 Active and passive green wall sites featured significantly higher fungal densities than the reference sites
201 across the three-month sampling period ($p = 0.001$ and $p = 0.009$ respectively; Figure 2). Temporal
202 differences in fungal density were not significant, nor were interactions amongst factors or with foot traffic
203 ($p > 0.05$). Despite elevated concentrations of aerosolised fungal propagules, total concentrations remained
204 well below the World Health Organisation guideline for indoor air [61] of 500 CFU/m^3 (Figure 2).

205



206

207 Figure 2: Average aerosolised fungal density (CFU/m^3) detected at active and passive green wall sites and
208 reference sites over the three-month sampling period. Error bars represent standard error of the mean.
209 Significant comparisons are indicated by asterisks ($p < 0.05$). Significant WHO Guidelines for safe indoor
210 fungal density is denoted with the dashed line at 500 CFU/m^3.

211

212 The frequency of generic occurrence, average propagule density and range of detection amongst sites are
213 displayed in Table 1. The most prevalent species was *Aspergillus ochraceus*, at a relative frequency of 83%
214 for the green wall sites and 39% for the reference sites, followed closely by the genus *Wallemia* at 82, 67 and
215 28% for the active, passive and reference sites, respectively. Despite *A. ochraceus* being the most frequently
216 detected species, the mean densities of *Wallemia* was higher at 40, 45 and 28 CFU/m^3, respectively.
217 Shannon's diversity index ranged between 1.24 and 1.98, where no significant differences were detected
218 amongst sites ($p = 0.098$) or through time ($p = 0.275$).

219

220 Table 1: Relative frequency, mean and range of density of aeromycota identified at active and passive green
221 wall and reference sites. Sample measurements across the three time points were pooled for this summary.

	Active			**Passive**			**Reference**		
Species	Frequency (%)	Mean (CFU/m^3)	Range (CFU/m^3)	Frequency (%)	Mean (CFU/m^3)	Range (CFU/m^3)	Frequency (%)	Mean (CFU/m^3)	Range (CFU/m^3)
Wallemia spp.	82	40	ND – 125	67	45	ND – 113	28	5	ND – 25
Aspergillus ochraceus	83	22	ND – 63	83	30	ND – 63	39	8	ND – 63
Penicillium spp.	58	16	ND – 113	58	10	ND – 38	22	3	ND – 25
Paecilomyces spp.	42	14	ND – 75	8	1	ND – 13	22	3	ND – 13
Aspergillus terreus	33	6	ND – 25	33	11	ND – 63	11	2	ND – 25

Sterile mycelia	25	4	ND – 25	25	6	ND – 38	11	3	ND – 25
Basidobolus spp.	17	3	ND – 25	50	7	ND – 25	33	6	ND – 25
Epicoccum spp.	17	3	ND – 25	*ND*	*ND*	*ND*	11	2	ND – 25
Cladosporium spp.	8	2	ND – 25	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
Rhodotorula spp.	83	1	ND – 13	8	1	ND – 13	11	1	ND – 13

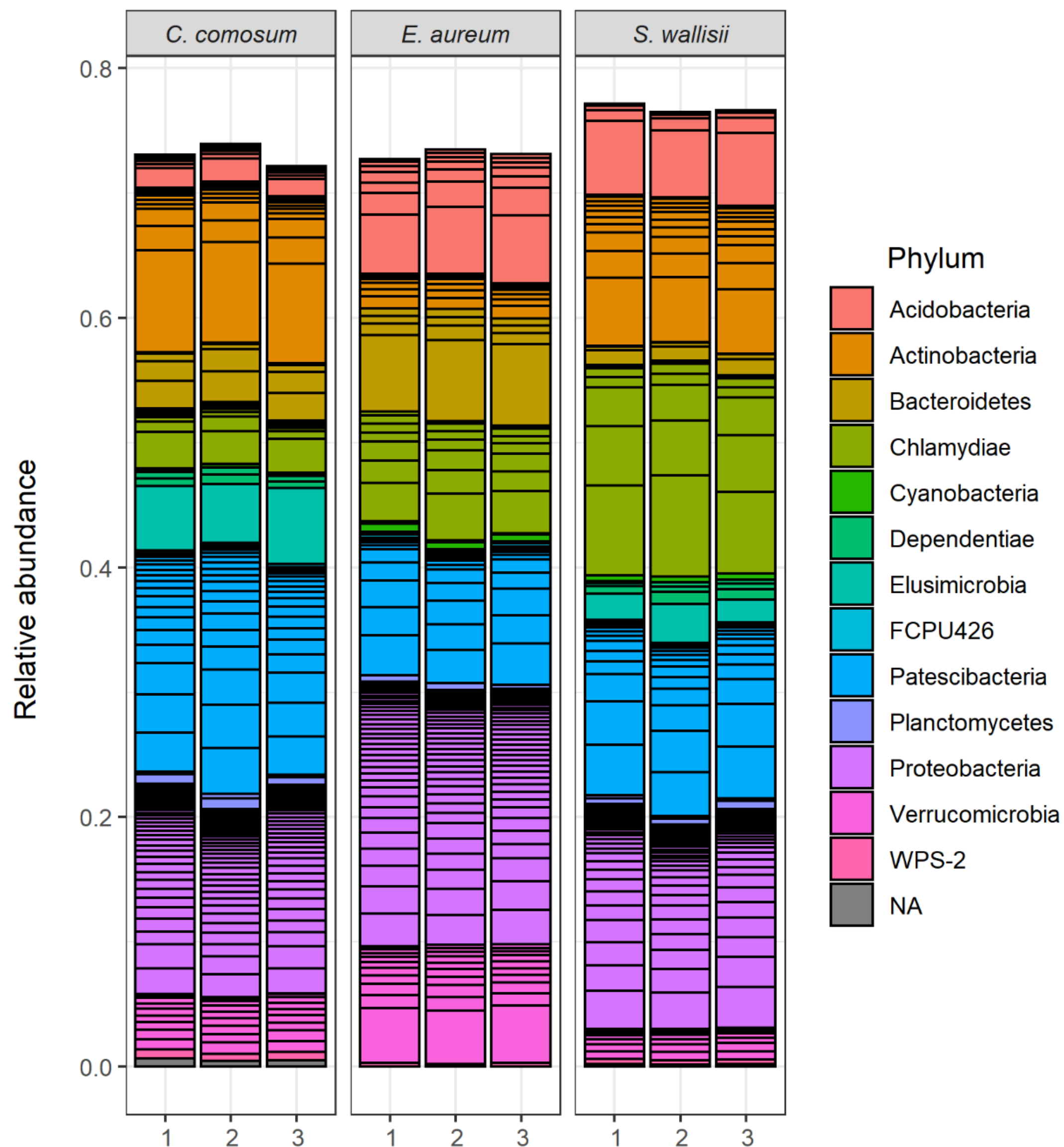
ND not detected

222

223

224 ### *3.2 Characterisation of substrate bacterial communities*

225 16S rRNA amplicon sequencing generated a total of 610,345 high quality reads, distributed across 2,825
226 Amplicon Sequence Variants (also known as zero-radius Operational Taxonomic Units, where differentiation
227 is made at a single nucleotide to avoid similarity-based clustering). These sequences were classified into 27
228 phyla, with the 120 ASVs that exceeded the gmRA (grouped mean relative abundance) cut-off accounting for
229 74.38% of the community composition (Figure 3). The bacterial community was dominated by Acidobacteria,
230 Acintobacteria, Bacteroidetes, Chlamydiae, Elusimicrobia, Patescibacteria and Proteobacteria, with these
231 phyla contributing to the ten most abundant ASVs (ASV1-10), and accounting for 24.9% of the total bacterial
232 community structure. In order of abundance, Proteobacteria contributed 26.7% of the total bacterial
233 community with 953 individual reads (ASVs), followed by; Patescibacteria (17.6%: 223 reads), Actinobacteria
234 (13.6%: 367 reads), Chlamydiae (12.1%: 206 reads), Acidobacteria (8.3%: 132 reads), Bacteroidetes (6.3%:
235 204 reads) and Elusimicrobia (2.6%: 11 reads).



236

237 Figure 3: Relative phylum abundance of bacteria associated with the three single- species botanical modules
238 from the active green wall site, showing technical triplicates. The 120 ASVs that exceeded the gmRA cut-off
239 across plant species are displayed (with 2705 ASVs excluded).

240

241 PERMANOVA identified significant differences in bacterial community structure among plant species (p =
242 0.005), which was confirmed graphically by an nMDS plot revealing distinct separation of the bacterial
243 community between plant species (Supplementary 1). SIMPER analysis attributed 64.6 – 72.3% of the
244 dissimilarities in bacterial community structure among the three plant species to four phyla (Table 2).
245 Differences between *C. comosum* and *E. aureum* were primarily driven by Patescibacteria (25.5% of the sum
246 dissimilarity between species); *C. comosum* and *S. wallisii* by Chlamydiae (20.8%); and *E. aureum* and *S.*
247 *wallisii* by Proteobacteria (19.7%).

248

249

Table 2: SIMPER analysis results, showing phylum contributions to amongst species differences in bacterial community structure from the three single-species botanical modules from the active green wall site. A dissimilarity contribution threshold of 10% was used for comparisons.

C.comosum - E. aureum		***C.comosum - S. wallisii***		***E. aureum - S. wallisii***	
Phyla	Contribution %	Phyla	Contribution %	Phyla	Contribution %
Patescibacteria	25.47	Chlamydiae	20.82	Proteobacteria	19.72
Actinobacteria	23.01	Proteobacteria	17.61	Chlamydiae	18.82
Proteobacteria	16.2	Patescibacteria	14.76	Actinobacteria	16.92
		Actinobacteria	11.84	Patescibacteria	16.88

Of the 2,825 ASVs sequenced, 33 individual reads were identified as members of the Legionellaceae family (Phylum Gammaproteobacteria), whereby 31 reads were assigned to the genus *Legionella* (Supplementary 2). Two ASVs (ASV303 and ASV1316) could not be identified to the genus level but were included as *Legionella* because the Legionellaceae contains only one genus. Interestingly, the distribution of the 28 least abundant *Legionella* reads differed between plant species.

3.3 Bacterial bioaerosol assessment

Aerosolised *Legionella* assessment yielded no indication of presumptive *Legionella* colonies with respect to the *Legionella pneumophila* positive control. Several unknown bacterial colonies grew on the modified RCS strips, but these were morphologically distinct from *Legionella* spp., and were thus not identified further.

264 # 4. Discussion

265

266 ### *4.1 In situ bioaerosol analysis*

267 While potting soils have been implicated as a source of human pathogens [62–65], studies documenting the
268 dispersal of aerosolised fungal pathogens from indoor contaminated soils is limited [66]. Several studies have
269 found that neither potted plants nor complex biowall structures contribute significantly to allergenic or
270 pathogenic airborne fungal density [66–70], unless considerable physical disturbance or agitation occurs
271 [71]. Nonetheless, as active green wall system circulate air through large volumes of biomass,
272 characterisation of these systems is prudent to assess the likelihood of unfavourable microbial growth and
273 the proliferation of fungal and bacterial aerosols.

274 Our results demonstrate that both active and passive green walls contributed significantly to ambient fungal
275 aerosol concentration in the tested environments relative to reference sites. However, viable propagule
276 counts at both walls remained well below the World Health Organisation maximum guidelines of 500 CFU/m^3
277 for indoor fungal spore loads [61], and are classified between very low (50 CFU/m^3) and low (200 CFU/m^3)
278 according to Singh *et al* [72]. A comparative assessment conducted on small scale active green walls by Irga
279 *et al* [66] hypothesised that the use of larger installations may increase the density of bioaerosols [66],
280 however, this was not the case in this study. The results presented here, while higher than those reported
281 by Irga *et al* [66], fell into the same low classification range [72].

282 No highly pathogenic fungal species were detected (e.g. *Aspergillus fumigatus*) in our analysis, and fungi with
283 known allergenic properties (*Aspergillus, Cladosporium, Epicoccum* and *Penicillium*) were measured in
284 concentrations below their proposed allergenic limits [73]. In line with previous green wall research assessing
285 ambient bioaerosols [66], no dimorphic or systemic pathogens were detected, nor were any dermatophytes.
286 Further, the species identified were comparable to previous active green wall studies in the area [66,74] and
287 generally consisted of common indoor fungi that do not represent health concerns for immunocompetent
288 people. This suggests that while green walls may contribute to an increase in the fungal loading of a space,
289 it does not change the fungal load composition.

290

291 ### *4.2 Substrate analysis*

292 Despite the highly variable diversity of bacterial species in soil environments, diversity at the phylum level is
293 remarkably stable world-wide [75]. The substrate bacterial communities detected in this study were similar
294 to previous studies on phytoremediation systems [76–78]. However, the bacterial community composition
295 varied significantly amongst plant species (Figure 3; $p = 0.005$) with Proteobacteria, Patescibacteria and
296 Actinobacteria driving differences amongst biofilters with different plant species (Table 2).

297 It is commonly accepted that rhizospheric bacterial communities are controlled by specific assembly rules
298 [79], where factors such as soil type, plant compartment, host genotype/species, plant immune system
299 behaviour, plant trait variation/developmental stage and residence time/season influence bacterial
300 community composition [79]. It is thus likely that differences in root structure and other plant traits are the
301 driving factors for bacterial variances amongst the plant species tested, as there were no other obvious
302 differences between green wall modules. As active green walls utilise a range of botanical species, variations
303 in their natural capacities to host different bacterial communities may, in future, influence their relative
304 success as indoor air phytoremediators.

305 The prevalence and distribution of Proteobacteria was consistent with earlier rhizospheric studies [1,78,80–
306 82], where Alpha- and Gammaproteobacteria were of almost equal distribution. Within these two classes,
307 the *Acetobacteraceae* (Alpha-) and *Burkholderiaceae* (Gama-) contributed 11.54% and 11.69% of the total

308 Proteobacterial density respectively. These findings align with those of Russel *et al* [1] and Mikkonen *et al*
309 [78], who performed substrate analyses of field-deployed botanical biofilters. Further, the potential VOC-
310 utilising families identified by Mikkonen *et al* [78] (*Nevskiaceae, Patulibacteraceae* and *Xanthobacteraceae*)
311 were also identified in the current study. Interestingly, the genera *Devosia, Prosthecomicrobium* and
312 *Hyphomicrobium*, which are VOC degraders that were found to be abundant in both previous bodies of work,
313 were largely underrepresented in the current study [1,78]. Active green walls equivalent to that tested here
314 have been previously shown to be highly effective VOC remediators [41].

315 The newly re-classified superphylum Patescibacteria has been estimated to encompass more than 15% of
316 the bacterial domain [83]. Due to their reduced genomic profile and limited metabolic potential,
317 Patescibacteria are believed to be plant-root symbionts [84–87]. In this study, Patescibacteria were
318 dominated by the order Saccharimonadales (56.6%), a vastly understudied group [88]. A recent study by
319 Lemos *et al* [87] found evidence that the Saccharimonadales possess uncharacterised metabolic mechanisms
320 that facilitate nutrient uptake [87]. This discovery may lend support to the theory that these bacteria are
321 endophytic bacterial symbionts in botanical substrates [88]. With little previous research on these bacteria,
322 we hypothesise that variation in Patescibacteria composition amongst plant species may be associated with
323 differences in plant root structure or molecular mechanisms, by which plants favour specific bacterial
324 endophytes [89].

325 Another driver of dissimilarities in the bacterial communities amongst plant species was the Actinobacteria,
326 a widely distributed environmental taxon, ranging from soil inhabitants (e.g. *Streptomyces* spp.), plant
327 commensals (e.g. *Leifsonia* spp.), nitrogen-fixing symbionts (e.g. *Frankia* spp.) and cellulose metabolisers
328 (*Cytophaga* and *Sporocytophaga* spp.), to animal and plant pathogens (*Mycobacterium* spp.) [90–92].
329 Approximately 60% of biologically active compounds released in soils are attributed to this taxon, which are
330 noted for the production of plant-growth promoting chemicals [93,94]. For this reason, they are often
331 considered when developing phytoremediation bioaugmentation strategies for pollutants such as heavy
332 metals or pesticides [77,91,93]. Additionally, several other rhizospheric or endophytic Actinobacterial
333 families (Chitinophagaceae, Microbacteriaceae, Solirubrobacteraceae, Sphingobacterium, and
334 Streptosporangiaceae) were detected in comparable concentrations. These families have known properties
335 that may contribute to plant health, environmental sensing, disease regulation or drought resistance
336 [77,92,95,96].

337 There have been few studies that have characterised the microbial population of deployed commercial
338 botanical biofilters [1,78]. Mikkonen *et al* [78] building on the work of Russel *et al* [1], hypothesised that the
339 genus *Hyphomicrobium* may be a global green wall system inhabitant [78], however, this genus was not
340 detected in this study. Subtle differences in rhizospheric community structure may be attributed to a range
341 of factors such as growth substrate composition, geographical location, or plant species selection, and it is
342 likely that the rhizospheric community structure of botanical biofilters is more dynamic than previously
343 theorised.

344

345 ### ***4.3 Legionella contamination analysis***

346 While there is a growing body of research that validates the safety of active green walls regarding their
347 contributions to the indoor aeromycota [15,29,30,66], there is less evidence related to the emission of
348 pathogenic bacterial aerosols such as *Legionella* [97]. In Australia, most cases of legionellosis have been
349 attributed to sporadic environmental contamination by either *L. pneumophila* or *L. longbeachae*. However,
350 several major outbreaks by *Legionella pneumophila* sourced from building cooling towers have been
351 documented [37]. In the natural environment, *Legionella* spp. account for less than 1% of the total bacterial
352 community structure [28], which aligns with the levels detected here (Supplementary 2). While the density
353 of *Legionella* spp. detected would be considered low, due to the limitations of 16S Illumina Sequencing, this
354 study was unable to speciate the legionellae identified, and thus determine the extent of potential

pathogenic species/strains. It is thus suggested that future work makes use of species-specific qPCR to expand on the work conducted here.

Due to the botanical nature of these systems, strict watering regimes should be employed by service providers. The transmission of many pathogens is aerosol dependant [37,98], and it is therefore essential that biofilters are maintained at an optimal water content which is sufficient for normal plant operations, but does not allow water to stagnate within the substrate or harbour biofilms in irrigation catchments [99–101]. In addition to the low concentrations of *Legionella* reported, this active green wall system employed the use of gravimetric drip irrigation – a system that lacks the pressurised components that may lead to active aerosolisation [33,34,38] – making the risk of pathogenic dispersal very low, provided the systems are well maintained.

Briefly, several additional potential pathogens were identified with 16S Sequencing (Supplementary 3), however the relative abundance of these bacteria were insufficient to warrant concern [102].

5. Summary and Conclusion

Fungi are ubiquitous soil inhabitants and have strong associations with plants. The installation of botanical material indoors, either as simple potted plants or complex active green walls, is likely to contribute to the ambient fungal load [29,70,103,104]. Fungal aerosols in the ambient indoor environment proximal to active and passive green walls remained well below WHO guidelines and the systems did not release detectable harmful fungal bioaerosols. The concentrations detected were comparative to previously reported literature [28,29,32,66,68], where no pathogenic or allergenic species at symptomatic concentrations were detected.

With an increase in commercial interest in this technology, there is the potential for companies to rush products to market without acceptable research into design and maintenance. In these instances, there is the possibility for poorly maintained systems to provide the niche environments required for an increase in fungal propagation or the formation of harmful bacterial biofilms. In order to prevent this, further scientific research is required on the factors that may influence dispersal of fungal propagules from green technologies. Additionally, more field studies are required of deployed commercial systems in order to validate the research findings presented here and in previous green wall literature.

Whilst the building tested in the current work was new and built to a very high standard, with a concomitant low background fungal diversity, it is possible that less well-maintained structures may experience different effects from the installation of green infrastructure. It is not possible from the current findings to determine whether green walls would make a greater or smaller contribution to the bioparticle load of a building with a higher background level of contamination, and it is recommended that this issue be addressed in future research.

Phylogenetic distribution of bacterial species provided insight into the composition of the bacterial community in a commercial botanical biofilter deployed in the southern hemisphere. These results differed in some aspects from those previously reported [1,78], inferring that the composition of microbial communities associated with green wall systems may be governed by region, substrate or plant species.

Further, no aerosolised *Legionella* was detected over the three-month monitoring period. Both the abundance of *Legionella* and the pressurised mechanisms required for aerosol dispersal were absent in these systems, and therefore present little risk of a contamination event, provided the systems are maintained at the industry specifications.

This body of work contributes a unique perspective to the microbial state of botanical biofilters situated in the southern hemisphere. The contribution of green walls to the ambient fungal load has been documented, however the bacterial dynamics of these systems is largely understudied. Further work in this area should

399 aim to address the bacterial composition of the rhizosphere of deployed botanical biofilters *in situ*, as well
400 as the bacterial and fungal aerosols they may generate.

401

402 **Authors' contributions**

403 RF, PJI and FT designed the study; RF collected samples; RF and PJI analysed fungal samples; RF and NW
404 analysed bacterial samples; RG and RF analysed the data; RF interpreted data; RF, TP, RG, PJI, FT and JS
405 drafted the manuscript.

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408 the contribution of "Figure 1: Schematic representation of bioaerosol sampling locations across each green
409 wall site of a newly built commercial office building. Sampling was conducted over two stories, 1.8 m above
410 ground and 0.5 m from protruding vegetation at both sites.".

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413

414 # 6. References

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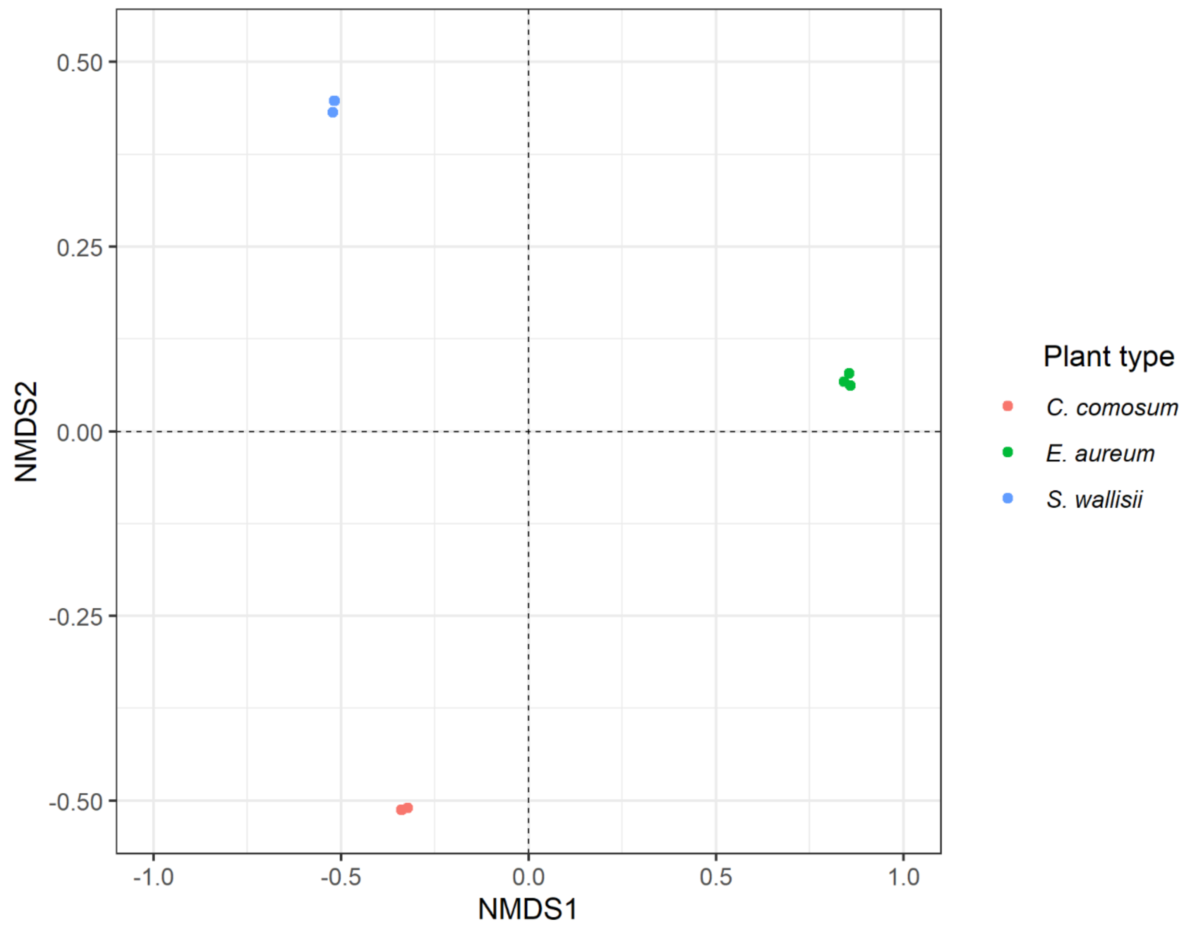
691 Supplementary



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693 Supplementary 1: nMDS plot showing dissimilarities in bacterial community structure amongst active green
694 wall plant species, with significant differences detected between species (PERMANOVA; $p = 0.005$; $R = 0.977$;
695 stress = < 0.01).

Supplementary 2: Average abundance (concentration/100 mL) ± SEM of individual Legionella ASVs for each plant species. Three ASVs (52, 108, 116) exceed the grouped mean relative abundance (gmRA) cut-off and were included in Figure 3. Percentage population abundance (PPA) is listed as function of its contribution to the total bacterial community composition (Legionella <1 % of total bacterial community).

ID	Genus	*C.comosum*	*E. aureum*	*S. wallisii*	gmRA	PPA (%)
ASV52	*Legionella*	22.9 ± 1.6	90.9 ± 32.5	4.2 ± 1.1	250.0	0.378
ASV108	*Legionella*	10 ± 0.9	42 ± 14.8	1.5 ± 1.5	112.2	0.17
ASV116	*Legionella*	45.1 ± 3.8	11.3 ± 4.4	17.7 ± 4.4	101.9	0.154
ASV224	*Legionella*	17.2 ± 2.8	4.4 ± 2	10.3 ± 2.8	43.4	0.066
ASV317	*Legionella*	9.1 ± 1	3.3 ± 1.2	7.1 ± 1.1	28.3	0.043
ASV439	*Legionella*		7.4 ± 2.6		16.6	0.025
ASV511	*Legionella*	5.9 ± 0.7	2.4 ± 0.9		12.1	0.018
ASV553	*Legionella*	2.5 ± 1.4		5.6 ± 1.9	10.1	0.015
ASV664	*Legionella*	0.9 ± 0.5	0.2 ± 0.2	4.2 ± 1.1	6.9	0.01
ASV802	*Legionella*	2.3 ± 0.5	0.6 ± 0.4		4.3	0.007
ASV844	*Legionella*			2.9 ± 0.6	4.0	0.006
ASV863	*Legionella*			2.9 ± 1	3.8	0.006
ASV940	*Legionella*			2.4 ± 0.7	3.1	0.005
ASV1259	*Legionella*	1.1 ± 0.6			1.2	0.002
ASV1279	*Legionella*			0.8 ± 0.6	1.2	0.002
ASV1281	*Legionella*			0.5 ± 0.5	1.2	0.002
ASV1317	*Legionella*		0.2 ± 0.2		1.1	0.002
ASV1321	*Legionella*			0.9 ± 0.9	1.11	0.002
ASV1348	*Legionella*	0.8 ± 0.8			1.00	0.002
ASV1412	*Legionella*		0.1 ± 0.1		0.89	0.001
ASV1554	*Legionella*		0.1 ± 0.1		0.67	0.001
ASV1594	*Legionella*			0.7 ± 0.7	0.67	0.001
ASV1616	*Legionella*	0.5 ± 0.5			0.56	0.001
ASV1619	*Legionella*	0.5 ± 0.5			0.56	0.001
ASV1638	*Legionella*		0.3 ± 0.3		0.56	0.001
ASV1652	*Legionella*		0.1 ± 0.1		0.56	0.001
ASV1695	*Legionella*			0.6 ± 0.6	0.56	0.001
ASV2447	*Legionella*		0.1 ± 0.1		0.22	0.00
ASV2538	*Legionella*				0.22	0.00
ASV2580	*Legionella*		0.1 ± 0.1		0.22	0.00
ASV2669	*Legionella*			0.2 ± 0.2	0.22	0.00
ASV303	NA	1 ± 0.5	11.6 ± 3.2		29.89	0.045
ASV1316	NA		0.7 ± 0.4		1.11	0.002
Total	*Legionellaceae*	119.8 ± 55.56	175.8 ± 122.15	62.5 ± 35.32	–	0.969%

700 Supplementary 3: Additional bacterial genera (identities/100 mL ± SD) containing opportunistic human
701 pathogens described by Baron *et al* [102]. Relative abundances presented here are insufficient to warrant
702 concern or sterilisation. [102].

ID	Class	Genus	*C. comosum*	*E. aureum*	*S. wallisii*	gmRA
ASV75	Actinobacteria	Mycobacterium	12.7 ± 2.4	1.3 ± 1.1	122.6 ± 27.9	181.4
ASV134	Actinobacteria	Mycobacterium	5.2 ± 1	1.1 ± 0.9	54.1 ± 13.5	81.2
ASV268	Actinobacteria	Mycobacterium	5.5 ± 1.3	7.2 ± 2.9	9.9 ± 2.1	35.4
ASV355	Actinobacteria	Mycobacterium	12.7 ± 0.8	0.7 ± 0.5	5.9 ± 2.7	23.9
ASV495	Actinobacteria	Mycobacterium	3.6 ± 2.1	3.0 ± 3.0	3.2 ± 3.2	12.6
ASV656	Actinobacteria	Mycobacterium		3.7 ± 1.7		7.1
ASV728	Actinobacteria	Mycobacterium			3.4 ± 2.0	5.7
ASV759	Actinobacteria	Mycobacterium			3.7 ± 0.6	5.1
ASC880	Actinobacteria	Mycobacterium	2.9 ± 1.5			5.1
ASC954	Actinobacteria	Mycobacterium	2.5 ± 1.3			2.9
ASC981	Actinobacteria	Mycobacterium	2.2 ± 1.1			2.7
ASC1079	Actinobacteria	Mycobacterium			1.6 ± 1.1	2.1
ASC1220	Actinobacteria	Mycobacterium			0.8 ± 0.5	1.4
ASC1318	Actinobacteria	Mycobacterium		0.2 ± 0.2		1.1
ASC1697	Actinobacteria	Mycobacterium			0.6 ± 0.6	0.6
ASV385	Alphaproteobacteria	Sphingomonas	14.2 ± 0.4		3.4 ± 1.1	21.0
ASV782	Alphaproteobacteria	Sphingomonas	2.1 ± 2.1	1.2 ± 1.2		4.7
ASV987	Alphaproteobacteria	Sphingomonas	2.1 ± 2.1			2.7
ASV1084	Alphaproteobacteria	Sphingomonas	1.9 ± 1.9			2.0
ASV1299	Alphaproteobacteria	Sphingomonas	1.0 ± 1.0			1.1
ASV1674	Alphaproteobacteria	Sphingomonas			0.4 ± 0.4	0.6
ASV561	Bacteroidetes	Chryseobacterium	8.7 ± 2.5			9.9
ASV644	Bacteroidetes	Chryseobacterium			5.6 ± 1.4	7.4
ASV742	Bacteroidetes	Chryseobacterium	4.6 ± 0.6			5.3
ASV890	Bacteroidetes	Chryseobacterium		0.2 ± 0.2	2.5 ± 1.0	3.6
ASV1350	Bacteroidetes	Chryseobacterium	0.8 ± 0.8			1.0
ASV2237	Bacteroidetes	Chryseobacterium	0.2 ± 0.2			0.2
ASV470	Gammaproteobacteria	Pseudomonas		4.4 ± 1.3	2.8 ± 1.1	14.2
ASV623	Gammaproteobacteria	Pseudomonas		3.9 ± 1.6		8.1
ASV1135	Gammaproteobacteria	Pseudomonas		0.6 ± 0.4		1.8
ASV1257	Gammaproteobacteria	Pseudomonas	1.1 ± 1.1			1.2
ASV959	Gammaproteobacteria	Acinetobacter		1.2 ± 0.3		2.9
ASV2045	Gammaproteobacteria	Acinetobacter				0.3
ASV529	Gammaproteobacteria	Stenotrophomonas	9.4 ± 0.1			10.9
ASV2587	Gammaproteobacteria	Stenotrophomonas		0.1 ± 0.1		0.2

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