

1 **C. Morris (Referee)**

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4
5 *[A0]* For clarity and easy visual distinction, the referee comments are copied here in black preceded
6 by bracketed, italicized numbers (e.g. *[1.1]*) to itemize individual comments or questions. Authors'
7 responses are offset in blue below each referee statement with matching numbers (e.g. *[A1.1]*). Page
8 and line numbers refer to online ACPD version.

9
10 *[1]* GENERAL REMARKS The authors have presented extensive details about aerosol
11 composition and concentrations following rain events. The data are interesting and
12 their publication would be a service to the scientific community. The extent of their
13 impact on our understanding of the interaction of land surface covers, bioaerosols and
14 atmospheric processes is not as palpable as the authors claim because some important
15 details are missing in the presentation of the materials and methods and in the
16 results. In the current version of the manuscript, the description of the experimental
17 set-up, in terms of the exact placement of samplers and environmental probes in the
18 forest canopy, is incomplete and makes it difficult to evaluate some of the phenomena
19 observed and how they are related to atmospheric phenomena on a broader scale. Re-
20 sults of the extensive microbial analyses could be presented in more detail to allow the
21 reader to evaluate the validity of the conclusions. The conclusion should be strengthened
22 to better highlight the novel conceptual contributions of this work and how it will
23 boost our understanding of the link between biological ice nucleators and rainfall.

24
25 *[A1]* In response to the referee's helpful comments we have strengthened the conclusions, added to
26 the material and methods section by describing methods and sampler placement in significant
27 additional detail, and improved discussion of microbial methods. Specific responses are outlined
28 below.

29
30 *[2]* SPECIFIC REMARKS P 1769-1770 L 26, 1-2: The authors should give more background
31 information to explain the logic of their argument. They state that "however,
32 evidence linking bioaerosols with increases in IN, especially : : : following precipitation,
33 is limited". Why would they expect there to be a link with IN after precipitation? Although
34 this has been observed several times, perhaps it is surprising? A little bit more
35 lead-in information would be useful.

36
37 *[A2]* Indeed, observations linking precipitation and IN have been published as have observations
38 linking precipitation and bioaerosol release. Only the Constantinidou et al. (1990) work discussed
39 ice-active bioaerosol during or following rain, however, and this was only for one species of
40 bacteria. Thus, the observations here represent a significant addition to the scientific literature. For
41 clarification the following text has been added:

42
43 "For example, splash-induced emission of fungal spores during rain has been well documented (e.g.
44 Hirst, 1953; Allitt, 2000). Bigg and Miles (1964) observed a correlation between rainfall and IN
45 concentrations when comparing extensive measurements in Australia, and Constantinidou et al.
46 (1990) observed a downward flux of ice-active *Pseudomonas syringae* bacteria during rain.
47 However, real-time measurements directly linking ambient bioaerosols and ice nuclei have been
48 missing."

49
50 *[3]* P 1770 L 3-4: The authors state "there is an apparent disconnect between concentrations
51 of IN active biological particles commonly found on vegetation and concentrations

52 in the air above them (Garcia, et al., 2012)”. However, Lindemann et al (1982) were
53 able to associate atmospheric bacterial concentrations to those of IN. The technique
54 that they used allowed them to detect 0.001 bacterial cells /L of air. This level of detection
55 was needed because INA bacteria were a very small fraction of the total bacteria
56 and were present at concentrations very near the detection threshold. In the work of
57 Garcia et al, their level of detection for INA bacteria was 0.1 gene copies /L. At 1 gene
58 copy per bacterial cell, this means that they could only detect 0.1 INA bacterial cells
59 /L of air. Hence, in order for them to have detected INA bacteria, the concentrations
60 under their sampling conditions would have needed to be 100 times those observed by
61 Lindemann et al. Hence the “disconnect” that they describe is most likely a problem of
62 sensitivity of detection.

63
64 **[A3]** We think that the referee may have misunderstood what we meant to imply regarding the
65 “apparent disconnection.” The goal of the Garcia study was to assess whether the vegetation studied
66 was a potential and significant source of INA bacteria to the atmosphere. Their results showed that
67 INA concentrations were on the order of 10^5 - 10^7 g⁻¹ of vegetation on the ground, while airborne INA
68 bacteria were mostly below the detection limit, which was 0.04 L⁻¹. And so, although the detection
69 limit from the Lindemann et al. (1982) paper that the referee discusses may have been superior to
70 that of Garcia et al. (2012), the point remains that large populations of INA bacteria on the ground do
71 not necessarily equate to large populations in the air above them.

72
73 To address the referee’s comments, the following text has been added to the main text at P1770, L5:

74 “For example, Garcia et al. (2012) recently reported that variations in the number concentrations
75 of airborne IN did not correlate with strong variations in the numbers of biological IN on underlying
76 vegetation.”

77
78 **[4]** P 1770, Materials and Methods. **[4.1]** In this section of the manuscript (including the relevant
79 information from the supplemental materials section) I cannot find information to allow
80 me to understand the position of the sampling devices relative to the plant canopy.
81 They state that the work was conducted in the Manitou Experimental Forest. **[4.2]** How
82 tall is the canopy and what is the leaf-area index (roughly)? **[4.3]** Is there an important
83 understory? **[4.4]** When rainfall is measured, is this the amount of rain observed on the
84 ground inside the canopy or elsewhere? **[4.5]** Likewise, in what part of the canopy was leaf
85 wetness measured, and how did they account for the variability within the canopy?
86 **[4.6a]** The authors state that inlets of samplers were placed at 4 m above the ground and
87 in the supplemental materials they state that they are at 1 – 4 m above the ground.
88 Given that they used the calculation of Lindemann et al for flux, then I suppose that the
89 1 m samples were used to calculate the difference in particle concentrations at the 2
90 heights. **[4.6b]** I don’t think that the assumptions of this calculation are met for measurements
91 within a canopy. The gradient method that they used for flux measurements has an
92 important assumption of fetch that I think could not be met under a canopy (generally
93 you need very large open fields with no obstacles to meet the assumption of fetch). **[4.6c]** The
94 authors seem to be suggesting that the measured flux is linked to emission from the
95 canopy, but can the measurements they made be extrapolated to what is happening
96 above the canopy? **[4.7]** In light of these remarks, Figure 5 is confusing and maybe even
97 misleading because it does not necessarily illustrate what they have measured.

98
99 **[A4.1]** A new figure (Fig. 1) has been added to the manuscript that details the location of inlets and
100 samplers.

102 [A4.2-A4.3] The following text has been added to address the referee’s comment regarding canopy
103 height, leaf-area index, and understory:

104 “The forest canopy at the site is sparse and highly variable. Clusters of ponderosa pine trees
105 approximately 10-20 m in height are separated by large open spaces of grass and forb understory.
106 Grasses are generally ~20 cm tall, with seed shocks occasionally reaching as high as 1 m. The leaf
107 area index (LAI) is also highly variable and was estimated to be a mean of approximately 1.9
108 (DiGangi et al., 2011), with trees covering ~60% of the site.” (Inserted at P1770, L25).

109
110 [A4.4] The following text was added to the main text at P1771, L6:

111 “Rainfall was measured in a clearing between tree clusters (Fig. 1) with a minimum of a 45°
112 sky view present at the precipitation monitoring sites. Therefore, summer precipitation
113 measurements were not affected by the tree overstory. Additional under-canopy precipitation
114 measurements were collected at the site but were not discussed in this manuscript. Those
115 measurements suggest that canopy interception within tree clusters is approximately 30% of the open
116 area precipitation when aggregated over the entire warm season.”

117
118 [A4.5] The following text was added at P1771, L11:

119 “The LWS was deployed approximately 1 m above ground in a clearing next to the precipitation
120 measurement sensor (Fig. 1).”

121
122 [A4.6a] Estimates of bioparticle flux were taken from the WIBS instrument operated from an
123 automated vertical profiling system running between 3 m and 22 m above ground. These details
124 were included in the online Supplement, but have been moved to the main text.

125
126 [A4.6b] The vertical profile was split into several sections, and a flux was calculated from the
127 gradient between these sections for data associated with each of the different clusters. This analysis
128 provided a large range of flux values immediately after rain events. While this wide range implied
129 the values are imprecise, the order-of-magnitude estimate was significant to share with the
130 community. As such, we have framed the stated range as a rough estimate rather than a canonical
131 calculation.

132
133 K-theory, or the flux-gradient method, is generally inadequate in dense forest canopies for use in
134 estimating fluxes from profile measurements, particularly where counter gradients in heat flux occur
135 in the trunk space and in thermally stratified conditions. Specifically, Monin and Obukhov Similarity
136 (MOS) theory (Monin and Obukhov, 1954) for wind and temperature do not hold, because the
137 Manitou Experimental Forest is not very dense. To correctly estimate a net flux would require a
138 more sophisticated inverse modeling approach (e.g. Siqueira et al., 2000), which we cannot provide.
139 However, recent measurements, Zhang et al. (2010), suggest that ϕ_m flux profiles can be described by
140 the local Richardson number, R_i , which increases linearly with ϕ_m in the canopy with height,
141 provided there is no counter gradient in sensible heat flux. The in-canopy derived momentum flux
142 profile (measured by the sonic anemometer profile) is used to derive this in association with the
143 WIBS concentration profile. The estimates listed here could be as uncertain as the aerobiology
144 approach adopted in climate models Sesartic et al. (2012), Jacobson & Streets (2009), but probably
145 better due to the inclusion of local momentum flux measurements.

146
147 [A4.6c] Regarding extrapolation to aerosol dynamics above the canopy, we cannot comment with
148 certainty about what happens to the aerosols after they are emitted from the canopy. As the referee
149 points out, we do not make the appropriate measurements for above-canopy flux estimates. It should
150 be noted that the uppermost measurements of the aerosol profile were above the top of the canopy,
151 allowing assertions to be made about flux between in and out of the canopy. Positive flux values

152 mean that aerosols, by definition, that are emitted from the canopy do not then re-enter. As such, one
153 might expect the aerosols to be transported away from the local area.

154
155 In response to the referee's comment about flux calculations, we have added the following text for
156 clarification (P1771, L26):

157 "Measurements within the canopy provide an upper limit for the role of biological IN in the
158 region above the canopy."

159
160 [A4.7] Finally, Figure 5 has been moved to the Supplement in response to the possible confusion it
161 could bring into the main text.

162
163 [5] If the work had been conducted over a low canopy of an annual crop, for example,
164 then the results would be relatively easy to interpret in light of experimental design.
165 But for the work as reported here, there is likely to be a very important impact of
166 canopy heterogeneity – both vertically and horizontally – on the measurements. When
167 rain hits the canopy top, much of it is likely to “dribble” down through the canopy after
168 the initial splashing, collecting particles as it successively comes into contact with
169 leaves. As pine leaves are very fine and hydrophobic, rain drops are likely to roll right
170 off. Secondly, there is often considerable litter in the understory, and when drops fall
171 on the litter, splashing of the abundant associated microflora will occur. Rainsplash
172 movement of spores within canopies can be very heterogeneous even for relatively
173 short canopies. Furthermore there have been considerable studies in this area, mostly
174 concerning the dissemination of plant pathogens. A few examples include: Paul et al
175 2004. Rain Splash Dispersal of *Gibberella zeae* Within Wheat Canopies in Ohio. *Phytopathology*
176 94:1342-1349. or, the very detailed chapter by Huber, Madded and Fitt on
177 Rain-splash and spore dispersal: a physical perspective, Chapter 17 in Jone D.G (ed.)
178 *The Epidemiology of Plant Diseases*, 1998 Kluwer Academic Publishers

179
180 [A5] There is no doubt that the processes involved in rain splash dispersal of bioparticles are
181 complex. We do not intend to suggest the precise mechanisms by which the observed particles were
182 emitted, and thus spend relatively little time on our proposed hypotheses within the manuscript. The
183 scope and conclusions of the manuscript are rather to show the larger scale observations regarding
184 the relationship between bioparticles, ice nuclei, and rain. And, as pointed out by the referee, there is
185 very well established literature showing rain splash as an important emission mechanism for many
186 types of spores and bacteria. In the submitted manuscript we had cited two of these (at P1776, L18).
187 We have added the citations suggested by the referee as well as several others for a total of ten
188 citations detailing previous work related to rain splash dispersal of bioparticles. As a part of this
189 change we have added the following text:

190 "The process of rain splash emission has been well documented and is the most likely source of
191 bioparticles observed, and several theories have been suggested to explain the micro-scale dynamics
192 involved (Hirst and Stedman, 1963; Leach, 1976;Allitt, 2000)."

193
194 [6.1] Hence, a considerable improvement to this manuscript could be made by the addition
195 of a figure that indicates precisely where in the plant canopy the measurements were
196 made. This figure could then indicate the scenario of the processes of rainfall impaction
197 and emission at the scale at which they were measured. It would add to the pertinence
198 of statements such as that in the Results and Discussion section concerning the origin
199 of the bioparticles observed after rainfall. [6.2] For example, on pp 1776-1776, lines 28-
200 29 and lines 1-7, the authors mention that bioparticles could have been emitted from
201 wetted surfaces near the measurement location. What does “near” mean? The forces
202 of agitation and wetting due to rainfall are known to release some spores on the scale of

203 centimeters. Are the measurements made within centimeters of these sources, and if
204 not then what trajectories and forces explain their abundance near the sampling inlets?
205 (I am not casting doubt; I am just asking for clarification.)

206
207 *[A6.1]* In response to the referee's first comment above we have added a new Figure 1, which shows
208 the relative locations of samplers and inlets. In addition we have added clarifying text to the
209 materials section that numbers each inlet in relation to the added figure, and the text now outlines the
210 specific heights from which each inlet sampled air.

211
212 *[A6.2]* The referee also asked for clarification of the proximity of the sampling point to the emission
213 point. We don't have measurements that detail the dynamics involved in the processes that moved
214 particles from the surfaces from which they were likely emitted to heights at which we were able to
215 sample them, and we don't claim to present a hypothesis on this process. The point here is to say
216 that, at the heights of our samplers (~a few meters) the concentrations of bioparticles and IN
217 followed the trends we discussed. Ultimately, the goal is not necessarily to understand the dynamics
218 a few centimeters from leaves, but to understand the overall air mass at "ground level" which could
219 be lofted upward.

220
221 *[7]* P 1171 last line: In the sentence, "Fluorescent particles (Nf) detected by the UV-APS
222 and WIBS can be regarded as a lower limit for the abundance of primary biological
223 aerosol particles", two things are unclear. Does "can be regarded as" really mean
224 "are" (in fact are)? If so, please say it directly. Secondly, if this is the lower limit for
225 abundance, it would be useful if the authors indicated precisely what this meant in
226 terms of the number of particles /m³, for example.

227
228 *[A7]* The terminology has been changed from "can be regarded as" to "represent" to clarify this
229 point. The degree to which these numbers are a lower limit is not well understood, but was
230 addressed to a certain extent by Huffman et al. (2012), as cited in the text.

231
232 *[8]* P1773, paragraph beginning on line 6: There is no mention of detection limits for microorganisms in
233 this work. In particular, for DNA analyses, how many "copies" (cells,
234 spores) of a given microorganism need to be present in one m³ of air in order to be
235 detected with these techniques?

236
237 *[A8]* Amplification of DNA was performed via the PCR method, which is generally very efficient
238 for amplifying the DNA of biological organisms. In theory even minute amounts, e.g. one molecule
239 DNA per organism, are sufficient for the identification of PBAP (e.g. Després et al., 2007). Thus, as
240 little as one DNA molecule of the organism in question deposited onto the sample can be detected if
241 PCR settings have been optimized. However, primer pair sensitivity can vary and some primer pairs
242 may need more than one molecule to initiate amplification. Additionally, DNA can be lost during the
243 extraction process, and this loss can vary as a function of experimental procedure (e.g. between
244 extraction kits). Although optimal sampling, extraction and amplification scenarios have been tested
245 previously, specific detection limits have not been rigorously studied.

246
247 For clarification, the following text was added:
248 "Although PCR can theoretically lead to detection of as few as an individual molecule of DNA
249 within a sample, actual results may be less efficient. Extraction and amplification scenarios have
250 been tested previously (Fröhlich-Nowoisky et al., 2009; 2012), but specific fungal and bacterial
251 DNA detection limits have are not yet available."

252
253 *[9]* P 1773, paragraph beginning on line 23: Other information is necessary to be able to

254 interpret the results and compare with other works. **[9.1]** For bacterial and fungal cultures,
255 how long were the cultures incubated? In the case of bacterial cultures, was the incu-
256 bation time sufficient to get the cells past the exponential phase of growth? **[9.2]** A second
257 very important piece of information that is needed concerns the densities of the suspensions
258 tested in each well. For bacteria in particular, this would allow comparison
259 with other reports concerning the number of ice nuclei/cell. This would also allow the
260 authors to compare the activity in the strains they characterized here: does a negative
261 reaction at -6_C, for example, for one strain have the same weight as a negative reaction
262 for another strain (in light of the fact that they might have been tested at different
263 cell concentrations). **[9.3]** Finally, what was considered as a positive result? Was a single
264 well frozen at a given temperature sufficient to be considered positive?
265

266 **[A9.1]** Some of this information had been discussed in the online supplement, but we have moved it
267 to the main text for clarification. The following text, for example, was moved to the main text into
268 what was Section 2.3 P 1773/L25 (now Section 2.5.3):

269 “Fungal and bacterial colonies were picked and cultured ... and incubated at 16 °C for 13-40 days
270 (first test) and 62-82 days (second test).”
271

272 And among additional information moved from the supplement:

273 “Ice active isolates were then cultured on DPY agar and incubated at 16 °C for ~ 3-7 days and
274 tested again for ice nucleation activity.”
275

276 **[A9.2]** The concentration density of bacteria or fungal material in suspension was not measured.
277 Additionally, the bacteria colonies were not picked intentionally, because the experimental procedure
278 had been optimized for fungal analysis. The referee’s comments are interesting, but will require
279 follow-up in an additional experiment. To clarify this point we have added the following text at the
280 beginning of Section 2.5.3:

281 “The intention was to pick select fungal colonies from the culturing media, but several bacterial
282 colonies were picked unintentionally as well.”
283

284 **[A9.3]** This information has now been added to the manuscript in Section 2.5.3:

285 “Isolates were considered as positive if they showed ice nucleation activity in all tests.”
286

287 **[10]** P1775, L 8-16: Here the authors mention that precipitation leads to increases in the
288 concentration of coarse aerosol particles not embedded in rain droplets. It would have
289 been interesting to know if these phenomena and the types of particles liberated are
290 specifically linked to rainfall per se or rather to the mechanical agitation caused by
291 rainfall. Interesting future experiments could involve supplemental mechanical agitation
292 during certain rainfall events and during certain dry periods. Can the authors speculate
293 about this based on existing literature concerning the role of mechanical forces in the
294 concentration of particulate matter in aerosols?
295

296 **[A10]** As the referee points out, indeed it would have been interesting to know the direct source of
297 the bioparticles observed during and after rainfall, and this work certainly opens additional question
298 that will be interesting to explore in future experiments. We refrained from speculating on
299 hypothetical possibilities we had no means of measuring, but rather decided to cite additional
300 previous work on bioparticles associated with rain.
301

302 **[11]** P1776 L 5-6. Here the authors make a statement about the diameter of the particles
303 related to the data presented in Fig 1e (Da) collected with the UV-APS. In the legend
304 in Figure 2 they again refer to particle diameter, measured with the same equipment,

305 but this time they call it aerodynamic diameter. This is confusing because for bioparticles
306 in particular the correlation between these two parameters (physical diameter
307 and aerodynamic diameter) is very poor (Reponen, T., et. al. 2001. Aerodynamic versus
308 physical size of spores: Measurement and implication for respiratory deposition.
309 Grana 40, 119-125). I think that the UV-APS device measures physical size of particles.
310 If this is the case the authors should make this clear and avoid using the term
311 aerodynamic diameter.

312
313 *[A11]* In this instance, the referee misunderstands the measurement parameter being discussed. The
314 UV-APS stands for ultraviolet aerodynamic particle sizer and only provides particle size as an
315 aerodynamic diameter (D_a), not as a physical diameter.

316
317 *[12]* P 1777 L 4-5: Here the authors state “suggesting a net upward flux of fluorescent
318 bioparticle emission after rainfall (50-500 /m/s)”. As mentioned above, it is crucial
319 to know the spatial pertinence of this estimate; where was flux estimated and is this
320 estimate representative of conditions that can be extrapolated above the canopy? It
321 would also be useful to see details about the flux calculations. They present the range
322 of values, but it would be interesting to see if in fact net flux after rainfall is always
323 upward and how variable are the measurements among all the replicates.

324
325 *[A12]* Please see author responses *[A4]* above. Additionally, aerosol gradient fluxes were calculated
326 using the WIBS4 located on the chemistry tower. We had intended this to be clear from text in the
327 supplementary material. However, we have clarified this point by adding the following text to
328 Section 2.3:

329 "The three minute WIBS4 descent profile data was split into several sections with height, and the
330 gradient flux calculated between each of these sections."

331
332 We assumed that the canopy concentration profile was homogeneous throughout the forest and so
333 this estimate represents a net vertical flux. The referee is asking for the "flux footprint". This can be
334 calculated using the appropriate Gaussian footprint model. The footprint will, of course, vary
335 significantly with atmospheric stability and height. Again, in order to avoid both introduction of
336 more uncertainty and over-interpretation of uncertainty estimates, we made the assumption of spatial
337 homogeneity when generalizing flux estimates to the wider forest.

338
339 We made an attempt to convey these fluxes while not encouraging their over interpretation by
340 characterizing them as such a wide range of values, i.e. 50-500 $m^{-2} s^{-1}$. We feel that expressing
341 variability between events in the paper would be overly verbose and would convey a lower level of
342 uncertainty than the analysis currently provides.

343
344 *[13]* P 1777, paragraph beginning on line 8. In this paragraph the authors indicate that
345 the identified microorganisms comprise a number of plant pathogens and human allergens.
346 Identification of bacteria was based on sequencing the gene for 16S rRNA. To
347 attribute a species name to the bacterium that is at the origin of the DNA, the authors
348 most likely compared the sequence to the NCBI data base (this is not described in the
349 methods). Likewise for fungi. These comparisons are associated with a probability
350 of similarity to sequences deposited in the data base. It is a common phenomenon
351 that sequences can be equally affiliated to several species. Likewise, many sequences
352 often do not find a significant match at the species level. In any case, the information
353 garnered from this approach only indicates the probability that the sequence resembles
354 something deposited in the NCBI data base (and most of what is deposited represents
355 soil habitats). This is why the details are very important here. How close were the

356 matches to the putative plant pathogens and human allergens? And did they match at
357 the species level or the genus level? A list of matches and their probabilities (at least of
358 list of the specific matches that correspond to well-known plant pathogens and human
359 allergens) would better substantiate their claim.

360

361 *[A13]* In response to the referee's comments we have added significantly more detail in Section
362 2.5.2, as detailed below (bold, italicized text added):

363

364 “Optimized methods of DNA extraction, amplification, and sequence analysis of the internal
365 transcribed spacer (ITS) regions *of genes* were used to determine fungal diversity from the high-
366 volume air filter samples (Fröhlich-Nowoisky et al., 2009; 2012). The primer pair ITS4Oo and ITS5
367 (Nikolcheva and Bärlocher, 2004) was used for amplification of *Peronosporomycetes* (formerly
368 *Oomycota*). Also specific for this study, the ITS regions from fungal lysates, obtained from the
369 cultivation experiments of Andersen impactor samples, were amplified with the primer pair ITS4 and
370 ITS5 (White et al., 1990; Fröhlich-Nowoisky et al., 2009; 2012). The obtained PCR products were
371 sequenced using the primer ITS5 and sequence analysis was performed as described previously
372 (Fröhlich-Nowoisky et al., 2009; 2012). *Summarized fungal sequences were compared with known
373 sequences using the Basic Local Alignment Search Tool (BLAST) at the National Center for
374 Biotechnology (NCBI) and identified to the lowest taxonomic rank common to the top BLAST hits
375 after chimeric sequences had been removed. Subsequently sequences that produced the same
376 BLAST results were pairwise aligned using the program BioEdit (version 7.1.3) and the similarity
377 calculated using the PAM250 Matrix. Sequences were grouped into operational taxonomic units
378 (OTU) when their similarity was > 97%. The sequences from the obtained operational taxonomic
379 units have been deposited in the GenBank database under following accession numbers: JX135610 -
380 JX136661 (fungi, and JQ976038 - JQ976273 (*Peronosporomycetes*).*

381

382 *For the determination of bacterial diversity from high-volume aerosol filter samples DNA was
383 extracted as described by Després et al. (2007). The 16S ribosomal gene was first amplified for
384 taxonomic identification with primer pairs 9/27f and 1492r (Weisburg et al., 1991) with PCR
385 conditions given by Després et al. (2007), and then cloned and sequenced. The same primer pair
386 was used for the bacterial lysates obtained from Andersen sampler culture plates. For the
387 bacterial sequences OTUs were identified using the Mothur software package (Schloss et al.,
388 2009) and chimeric sequences excluded (using the Bellerophon program (Huber et al., 2004).
389 Representative sequences of the OTUs were aligned using the multiple sequence comparison by
390 log expectation (MUSCLE) package (Edgar, 2004) and thereafter manually checked. Sequence
391 identification was based on phylogenetic analysis. Representative sequences were included as well
392 as type-species of various bacterial groups. Phylogenetic trees were based on a neighbor-joining
393 algorithm based on Jukes Cantor corrected distances within the Phylip package (Felsenstein,
394 2005). Bacterial sequences are deposited in the GenBank database under the following accession
395 numbers: JX228219-JX228862.*

396

397 *Although PCR can theoretically lead to detection of as few as an individual molecule of DNA
398 within a sample, actual results may be less efficient. Extraction and amplification scenarios have
399 been tested previously (Fröhlich-Nowoisky et al., 2009; 2012), but specific fungal and bacterial
400 DNA detection limits have are not yet available.”*

401

402 Also, to clarify the general point of the referee we also modified the discussion text discussed
403 (P1777/L16) by the referee here (bold, italicized text added):

404

405 “The identified groups of microorganisms comprise a number of plant pathogens and human
406 allergens (mildew, smut and rust fungi, molds, *Enterobacteraceae*, *Pseudomonadaceae*), *though not*

407 *all species within these groups exhibit pathogenic or allergenic properties.* These findings provide
408 a possible rationale for reported enhancements of asthma and other respiratory diseases during rain
409 showers (Taylor and Jonsson, 2004; Dales et al., 2012). *Follow-up studies will be necessary to*
410 *investigate the possible relationships and mechanisms on a species level.”*
411

412
413 [14] Overall, the data concerning microbial characterization have been presented in too little
414 detail. The authors present these data to support important points about the processes
415 underlying increases in IN abundance after rain. They went through a lot of work to obtain
416 them. So it is unfortunate that more detail is not presented. It is also unfortunate
417 that they make a cursory presentation of the results that corroborate their underlying
418 hypotheses without allowing the reader to evaluate the strength of their conclusions.
419 Out of curiosity, I recovered some the sequences for bacteria that they deposited in
420 GenBank and Blasted them against the NCBI data base to see what types of bacteria
421 they might have encountered. The sequences that I looked at corresponded to
422 soil-associated bacteria such Streptomyces and Arthrobacter. This made me wonder
423 if the relative abundances of soil bacteria were considerably less after rain than during
424 dry periods. It would be very useful if the authors presented a more detailed analysis,
425 perhaps by pooling the data for events after rainfall and those during dry periods
426 and then presenting the relative frequencies of the different groups of micro-organisms
427 encountered.

428
429 [A14] Please see author response [A13] above. Additionally, the detailed data for fungi and bacteria
430 will be published in separate manuscripts, as fully unraveling the inter-sample variability on species
431 level and detailed comparison of species associated with different ecosystems goes beyond the scope
432 of this study.

433
434 In general this manuscript presented an overview of the observations that biological particles and IN
435 increased during and after rainfall. The focus is to bring the observations to the community of
436 atmospheric scientists. While more detailed microbiological detail would be interesting to a small
437 subset of the readers here, these data will be more appropriate in a follow-up to a journal with a
438 deeper biological focus. As an example of the importance of this, Referee #3 requested that we
439 decrease the amount of microbiological detail that we give in the manuscript, because it is already
440 “difficult to read” for a non-biologist. As a result we have chosen not to radically expand the detail
441 in these sections, beyond what we have now added to Section 2.5.2.

442
443 [15] P 1778, paragraph beginning on line 9: Here the authors describe increases in the
444 abundance of ice nucleators active at temperatures warmer than -15_C and the concomitant
445 increases in particle sizes that are suggestive of biological particles. In parallel,
446 they have isolated fungi and bacteria that are ice nucleation active in laboratory
447 tests. [15.1] This is precisely where it would be useful to know the rates of ice nucleation
448 activity per biomass of the microorganisms. This information is critical for the authors
449 to claim that these micro-organisms were present in the atmosphere at sufficient concentrations
450 so that they could have contributed to the observed increases in ice nucleation
451 activity. Toward the end of this paragraph the authors state: “Overall, the DNA
452 analyses of aerosol samples collected during rain events showed higher diversity and
453 frequency of occurrence for bacteria and fungi from groups that comprise IN active
454 species (Pseudomondaaceae; Sordariomycetes). Identification of both Pseudomonas
455 sp. and Sordariomycetes directly from IN samples collected using the CFDC during
456 rain shows conclusively that the biological particles were indeed active as ice nuclei.”
457 [15.2] It should be noted that most bacterial species in the very large Pseudomonadaceae

458 family ARE NOT ice nucleation active. INA is limited to strains of a few species within
459 this family. Likewise for the over 10000 species in the Sordariomycetes: only one or
460 2 are INA. Even among the species of Pseudomonas, only a few are INA. The recent
461 work of Joly et al (2013. Ice nucleation activity of bacteria isolated from cloud water.
462 Atmos. Environ. (in press)) revealed that in clouds only about 10% of the fluorescent
463 pseudomonads isolated from clouds had measureable INA. So the statement made by
464 the authors is not well-supported by their generalizations. It would be better to indicate
465 the specific species that contributed directly to this increase in INA and the rates
466 (concentrations) at which they were present in the air.

467
468 [A15.1] The referee asked for detail about the ice nucleation activity per biomass of the
469 microorganisms. This information is not readily available but is planned for future experiments.
470

471 [A15.2] It is true that ice activity cannot be determined at the species level, because only certain
472 strains are IN active. This is why we discuss results from the high-volume and Andersen samplers at
473 the family level. For example, the following text was copied from the manuscript discussion:

474 “Overall, the DNA analyses of aerosol samples collected during rain events showed higher
475 diversity and frequency of occurrence for bacteria and fungi from groups that comprise IN active
476 species (*Pseudomondaaceae*; *Sordariomycetes*).”
477

478 In contrast to this, DNA analyses of samples collected from behind the CFDC directly measure
479 particles that acted as IN within the instrument, and thus no assumptions are necessary. Particles that
480 formed ice crystals were collected onto a grid and analyzed with respect to their relative abundance
481 and type. So in this case, the statement as previously written is still defensible:

482 “Identification of both *Pseudomonas sp.* and *Sordariomycetes* directly from IN samples collected
483 using the CFDC during rain shows conclusively that the biological particles were indeed active as ice
484 nuclei.”
485

486 The genetic data was collected and preliminary results presented to support the results gained by the
487 UV-APS data to show an increase in IN abundance after rain. Please also see response [A14]. For the
488 specific comment given by the referee, no soil samples were taken at the sampling site to be able to
489 compare their composition and abundance before and after rain.
490

491
492 [I6] P 1779, Conclusions. The first statement is accurate in light of the results of the work
493 presented here. But, an added detail would be useful. To the phrase “bursts of bioparticle
494 emission and massive enhancement of atmospheric bioaerosol concentrations”, it
495 would be useful to delimit this increase in terms of space. Where is the massive enhancement
496 occurring? Is it above the canopy or within the canopy in the forest system
497 studied?
498

499 [A16] We added text clarifying that the bursts were observed within the canopy. The first sentence
500 of the conclusions now read (bold, italicized text added):

501 “Our observations indicate that rainfall can trigger intense bursts of bioparticle emission *within*
502 *the forest canopy* and massive enhancements of atmospheric bioaerosol concentrations by an order
503 of magnitude or more ...”
504

505 [I7] The second statement is something that has not been demonstrated here (in terms
506 of spread and reproduction), but it is something that is well-known in the field of plant
507 pathology, for example. In this light, it does not seem pertinent that it is a conclusion of
508 this study.

509
510 [A17] Several citations were added to clarify that the observation of microorganism dispersal was
511 not uniquely observed by our experiments:
512 "... (e.g. Hirst and Stedman, 1963; Fitt et al., 1989; Constantinidou et al., 1990; Paul et al.,
513 2004)."
514
515 [I18] Figure 5: This figure does not really add anything to the manuscript. It contains well known
516 information (pathogen spread, for example) and also does not present any specific
517 details that have not been presented in other figures in other publications about
518 the interaction of bioaerosols with landscapes and the atmosphere.
519
520 [A18] Figure 5 has been moved to the Supplement (now Fig. S1) in response to the referee's
521 suggestion.
522
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