

Point-to-point reviewers' response:

All the reviewer's comments (in **boldfaced red**) have been numbered sequentially. After each comment the authors report their answer indicating eventual modifications made in the revised version of the manuscript. Indicated line numbers are relative to the track change version of the manuscript (called "revised manuscript" from here onward) in order to ease the browsing of the manuscript itself and assess changes to the original text. Also, in the track change version of the manuscript, main changes have been highlighted with a comment in the form of "Rn-Cn" where Rn indicates the reviewer number (1-3) and Cn the comment number.

REVIEWER #1

1) P2L22: ": : some periods: : some land uses." Rather vague description doesn't help the reader in getting an overview of previous work, rephrase or delete

Following the reviewer's advice, the sentence at **P2 L23** of the revised manuscript has been removed.

2) P2L32ff: "But while Burrows : : ." The whole sentence should be rephrased to improve readability. The word "species" is used but it's not entirely clear if the authors mean microbial species.

Also it is unclear why "in reality" emissions are different from the results from Burrows et al.. If this is a conclusion based on the submitted work, it should rather be made in the conclusions chapter.

The sentence at **P3 L2 – 4** of the revised manuscript has been removed to avoid unclear statements

3) P3L13ff: The site description is too general (e.g. no species resolved vegetation or bare soil coverage). This is surprising given that the authors describe the existence of a large variability in emission fluxes "especially because of variation in vegetation across space (P3L3)". Was the grassland actively managed, e.g. grazed or mown during or in between sampling? Was the management comparable between different campaigns and study sites?

The experimental field was not intensively grazed and was not actively managed during the measurement campaigns with no mowing or irrigation. This information has been added at **P3 L18-19** of the revised manuscript.

4) P3L24: How does the flow rate translate to Reynolds Numbers. What are the resulting losses in the sampler's intake? Are losses biased towards larger or smaller aerosols?

The virtual impactor was designed following good design practices from (Marple and Olson, 2011). Literature data report a sampling efficiency ranging from 80 to 100 % for mildew spores (Schwarzbach, 1979) and calibration tests performed during the 2008-2010 campaign showed it to be capable of sampling aerosolized *P.syringae*. Considering its high flowrate it is operating at super-iso-mean-velocity and therefore sampling efficiency is expected to decrease for larger particles proportionally with the ratio between external wind speed and the Burkard's sampling speed (Brockmann, 2011). This information has been added to **P4 L3-8** of the revised manuscript.

5a-d) P3-4 Chapter 2.1: General questions regarding the employed micro-meteorological measurement technique:

a) Besides precise instrumentation for the concentration gradient, steady state conditions are the key restriction for applying k-theory/gradient measurements. Was the sonic data used to investigate steady state conditions, e.g. employ standard quality checks: stationarity etc. (Foken and Wichura, 1996)?

b) Results from detectors precision study (MRG) are visible in figure 3 only. It would be helpful to also present actual values and compare them to measured fluxes.

c) was scaling of measurement height by the zero-mean displacement height discarded in estimation of K due to the comparably small canopy?

d) Since the authors employed a fast 3d sonic anemometer, I would welcome the addition of a more data driven flux footprint and possible flow distortion evaluation besides the cited literature relationships the authors followed. Did you discard measurements from specific wind directions, e.g. situations where sensor inlets are located downwind from the tower structure?

A more detailed description on quality checks, footprint analysis and flow distortion has been added in a new paragraph to the Supplementary Material S1. **One figure has been added to the main text (Fig. 4).** To summarize here:

- a) the quality check (QC) flagging system from (Mauder and Foken, 2006) was employed with flags ranging from 0 (best quality) to 2 (fluxes to discard). Only 4.54% of half-hourly measurements had a flag 2 in the September 2015 campaign where EC and flux-gradient (FG) method were compared. Keeping or discarding those data didn't significantly change the convergence of the two methods, nevertheless we chose to exclude flag 2 data in the new version of the manuscript where all the analysis and figures now reflect such exclusion.
- b) a new figure will be added (Figure 4) reporting the relationship between counted CFUs, fluxes and MRG. The other figures will be re-numbered accordingly. The new figure has been inserted in the revised manuscript. The figure is introduced in the text at **P8 L5-6**
- c) the zero plane displacement was computed as two thirds of the average canopy height at 0.13 m, and then z was computed above such zero level. However, as the reviewer points out, the small grass height makes the computation quite insensitive to zero plane placement, but however it was considered.
- d) In the EC/FG comparison experiment, grassland footprint resulted the main contributor to the measured fluxes (about 50-60% contribution). The reviewer is correct about possible flow distortion: we decided to use the Integral Turbulence Characteristics (ITC) test to assess the goodness of turbulence data, that is embedded in the QC flagging system described above. Since no flag 2 data were detected when wind was blowing through the scaffolding, we can assume the absence of significant flow distortion

6) P4L23: "similar herbaceous species, such as: : " these species were not listed for study site 1.

The authors would kindly point out that the two major species were already indicated at P3 L16 of the original manuscript (now **P3 L18**).

7) P5L7: You assume that deposition is purely driven by gravitational settling? Are other means (e.g. negative gradient between vegetation canopy and atmosphere, interception, impaction) insignificant?

We thank Reviewer #1 and Reviewer #3 for this suggestion that allowed us to improve the modelling approach. A new version of the PLAnET model has been developed including interception and impaction effects together with gravitational settling, following a widely adopted model (Slinn, 1982). The whole model has been re-optimized and the Gompertz parameter (Eq. (2)) re-computed. The model results remain consistent with the previous version, with only small differences in the linear regressions between measured and modelled daily averages. For the 2008-2010 campaign the slope changed from 0.71 to 0.70, offset from 0.88 to 0.28 and r^2 from 0.59 to 0.54. For the 2015 campaign the slope changed from 1.05 to 1.31, the offset from 0.11 to -3.75 and the r^2 from 0.57 to 0.68,

yielding an even better correlation between measured and simulated net fluxes. The relationship between the newly computed deposition velocity and friction velocity for the campaign data remains under the Gillette et al. critical threshold (Gillette et al., 1974; Gillette et al., 1997) and therefore no bias should exist in the measured fluxes. In fact, no depositional considerations are made for example by Park et al. (2011) when applying gradient method to PM₁₀ fluxes. The new version of the model will be uploaded to MathWorks FileExchange and all the data and figures in the revised version of the paper will take in account deposition, impaction and interception. The revised version of the paper acknowledge the insertion of the new deposition model and its equations at **P5 L 23-27, P6 L 13-17, P6 L 23-30, P7 L1-6**

As for the negative gradients, these were never observed in the current dataset. However we agree that this should require additional investigation and this *caveat* has been included in the revised version of the paper (**P13 L 23-26**)

8) P5L18: Why did you use the Lighthart and Shaffer data to express observed fluxes as a logistic function of u_{*} ? What is the goodness of fit for m_1 , m_2 , m_3 and eq.2 in general? How does the fitting errors propagate in overall model uncertainty.

The Lighthart and Shaffer data were used to parameterize Eq. (2) independently from the validation data. Using our own measurements to obtain a fit between u_{*} and fluxes would have probably resulted in better overall results when comparing the model to the data, but reduced the applicability of the model outside the presented situation. m_1 , m_2 , m_3 are the result of an iterative numerical optimization (this clarification has been added at **P6 L6**), therefore their confidence interval could not directly be computed; we performed a sensitivity analysis to assess the impact of those coefficients on fluxes (Table 2), revealing that changing the coefficients by 10% has a small overall impact on the predicted fluxes (the delta in the error function ϵ is < 0.5). The final adjusted r^2 was 0.44.

9) P6L5: The settling velocity is highly dependent on particle diameter and shape. Obviously for the applicability of a model, simplifications have to be made here, since time resolved aerosol size and density spectra are hard to measure or predict. However it would be worth exploring if deposition (i.e. V_g) has a larger impact on overall predicted net fluxes by varying aerosol size modes and densities (in reality these will have temporal patterns for instance due to phenology of different sources). It should at least be specified if the used literature values for particle density and particle diameter are representative only for grasslands or a specific season.

We refer to Raisi et al. (2013) that, even if related to a suburban area, is one of the most recent works where a long term monitoring of bacterial and fungal species was made and coupled with aerodynamic considerations. The seasonal and spatial variation that the reviewer's points out is absolutely true and is acknowledged as well by Raisi et al. (2013). As the reviewer correctly states, a single diameter choice was made as a simplification. In the cited work the highest fraction of cultivable fungi and bacteria was found in the range between 2.1 and 3.3 microns and we have therefore chose 3.3 μm as a cautionary representative diameter for bioaerosols in the model. The text has been modified to acknowledge the non-universality of this aerodynamic choice (**P11 – L18-21**).

10) P6L10: eq. 6: please report goodness of fit for linear regression between avg C and LAI. How does the uncertainty in C_a propagate into prediction of F_d ?

Goodness of fit between the variables is rather low ($r^2 < 0.2$), however its impact on the overall model and the predicted net fluxes is limited: a 50% increase in simulated concentrations (C_a in the model) resulted in an average percentage change of net fluxes of roughly 2% on the calibration dataset.

11) P8L5: The comparison between flux gradient and eddy covariance flux measurements provide confidence in the observations. However, eddy fluxes are not necessarily the truth, flux errors in LE are often in the magnitude of tens of percent. EC measurements were made at

different height than the gradient measurements, meaning that the EC instrumentation sees different parts of the grassland. Along these lines, the open path EC sensor was not cross-calibrated with the closed path gradient sensors? In other words, It will be hard to conclude if the gradient or the EC results are off. A more detailed error/uncertainty discussion of the net fluxes obtained from the gradient method would be appreciated here. It should at least be acknowledged that the conclusions made from the H₂O flux comparisons do not necessarily apply to aerosol flux measurements. What are the expected uncertainties introduced through assuming scalar similarity? The MRG or precision of the detectors employed at same height could be used to propagate a flux uncertainty. Since the model is calibrated on the measured fluxes (plus minus uncertainty) also the model will have this uncertainty.

We fully agree with the reviewer in that the good correspondence between EC and FG water vapour fluxes does not necessarily apply to bioaerosol flux measurements and we will add a corresponding note of caution to the revised manuscript (**P14 L30-32, P15 L1**)

As far as the concentration profile follows M-O theory, the difference in sampling height should not impact retrieved gradient fluxes. Sampling height is an input to the method and is therefore accounted for. Our field measurement conditions overall meet M-O requirements (e.g flat terrain, homogeneous).

The reviewer is correct that flux footprints from the two methods are likely slightly different, being larger for the EC that has a higher sampling height. However, the footprint analysis we performed revealed that majority of the EC footprint is contained within the experimental field, that is very homogeneous, making the two flux measurements safely comparable. Such discussion will be added to the revised version of the supplementary material.

The comparison between EC and FG was performed in order to assess the presence of a significant bias between the two methods (i.e.: significant under or over estimation of the FG method at low/high fluxes). The latter intent has been clarified in the revised version of the paper by modifying **P9 L8-9** and, in the conclusions, **P14 L30-32** and **P15 L1**.

12) P8L15ff: the 2008/2009 measurements have a wider spread, partly due to the fact that no detection limit was applied (e.g. in 2015 all negative fluxes were removed due to the detection limit). What is the reason for that?

In the 2008-2010 campaign, the samplers were calibrated with multiple replicates of aerosolized *P. syringae* at different dilutions (three dilutions at 10², 10³ and 10⁴ bacteria ml⁻¹, three replicates per dilution). Given that no statistically significant differences were detected between the two samplers (except in one replicate at 10³ bacteria ml⁻¹), no MRG was computed.

This explanation has been added to the revised version of the manuscript (**P5 L2-6**).

13) P11L33ff: besides rainfall other events could have an effect on PBA production. You mentioned the heat wave in 2003. What about water stress or cutting/mowing/ grazing. Some of these stress factors might have lagged interactions with LAI and microorganism population growth. It would be great to introduce 1 or 2 sentences about these effects and how they would change the annual emission from a grassland, if feasible

Following the reviewer comment, some further effects on PBAs production have been discussed in the revised version of the paper (**P14 L18-26**).

14) Fig(3): Why is the detection limit (MRG) half in Sept-Oct as compared to July?

We apologize for any lack of clarity. Figure 3 does not report MRG values, only fluxes. MRG values are calculated on the CFUs measured by the samplers and when the two samplers fall below such detection limit, the flux derived by FG method at that time is flagged (yellow star) as unreliable. The new figure 4 (see also comment 5b) should clarify the latter point

15) Technical Corrections: P1L19: “: : than that: :”. Numerus P3L12: “similar terrain”. Phrasing: Do you mean flat? P8L7: delete “,instead,” P8L7: rephrase, e.g.: spanned over multiple seasons P9L32: “: : all the emission-deposition processes”, please rephrase P12L14: “: : has been”. Numerus P12L21: “: : outward fluxes”. Rephrase, e.g. emission fluxes

All the technical corrections have been addressed in the revised version of the paper where indicated

REVIEWER #2

1) Discussion: Please include comparison between the PLaNET estimates and the previous studies, e.g. the Burrows et al, (2009b) and microbial flux observations from other locations, for instance using the same scaling factor to total microbes as Burrows et al. (2009a) used for grasslands (302).

We would like to thank the reviewer for this comment since it allowed us to explore potential convergences between PLaNET and ECHAM5. In fact, by looking at figure 4 in Burrows et al. (2009) the authors were able to extrapolate a median value for flux of total microorganisms for grassland (as simulated by ECHAM5) of roughly 1000 organisms $\text{m}^{-2} \text{s}^{-1}$. By scaling the PLaNET model outputs for the 2008-2010 and 2015 simulations with the factor proposed by the reviewer an average net flux of 750.49 organisms $\text{m}^{-2} \text{s}^{-1}$ was found. The latter estimate is referring to the new version of the PLaNET model including the new deposition scheme (see response to reviewer #1 and #3) and is a surprising result, considering the fact that PLaNET is still in its infancy. These comparisons, along with the relative considerations, have been added to the revised version of the paper (**P12 L15-22**).

2) Page 11, first paragraph suggests that obtaining a scaling factor to total microorganisms from the culturable fraction requires flux measurements of the total microorganisms. Why cannot the scaling factor be estimated from lower temporal resolution concentration measurements of both total microbes and the culturable fraction in the same site the flux measurements are made?

It would indeed be possible assuming that the culturability of airborne microorganisms does not change during the time span of the “slower” total microorganisms samples. The latter possibility has been added to the text as a suggestion for future experiments (**P12 L33-34 and P13 L1-2**).

3) Page 11, lines13-18: Culturable and viable are not necessarily the same thing (see e.g Burrows et al, 2009a)

In the revised version of the paper term “viability” in **P12 L25** has been changed to “culturability” and it has been clarified that epifluorescence, contrary to plate incubation, may detect viable-but-nonculturable microorganisms by adding a short sentence immediately after (**P12 L25-27**).

4) Page 2, line 20-23: "In the past, only few attempts have been made to quantify the flux of microorganisms from plant canopies (Lindemann et al., 1982; Lindemann and Upper, 1985; Lighthart and Shaffer, 1994) covering only some periods and some land uses." This is confusing, as later the authors themselves reference several other studies that have tried to quantify the fluxes using various methodology. Please specify that this sentence refers to direct measurements of bacterial fluxes.

The suggestion of the reviewer has been implemented by changing the sentence “In the past, only few attempts have been made to quantify the flux of microorganisms from plant canopies” to “In the past,

only few attempts have been made to directly measure the flux of bacteria” (P2 L20-21 in the revised version).

5) Page 4, line 23-24 “The experimental field for these previous campaigns was covered with similar herbaceous species such as cocksfoot (*Dactylis glomerata*), ryegrass, tall fescue (*Festuca arundinacea*) and alfalfa (*Medicago sativa*).” The list of species for the other field on the previous page only included clover and ryegrass, so how similar was the vegetation on these fields?

We apologize for the lack of clarity. The sentence has been corrected with “Herbaceous species with similar habitus” in the revised version of the paper (P4 L34).

REVIEWER #3

1) Some equations contain errors and units are missing or incorrect for a number of parameters, see the specific comments below. There are also a number of inconsistencies between the equations in the MS and in the code. I am puzzled most by the formulation for microbial population growth: is it assumed to respond instantaneously to changes in driving variables (temperature)? If so, is that a valid assumption on the 30 min. time step that you applied here, and at which time step would this assumption brake down? Or are the dynamics of the microbial population calculated transiently? Moreover, the formulation and units of eq. 8 are inconsistent, which is where most of my confusion comes from.

In the model it is assumed that the microorganisms grow by a temperature-dependent factor (r) each 30 minutes (1800 s). Relatively fast growth are not unheard in field samplings with doubling times reported as low as 3.5 to 3.8 hours (see Hirano and Upper (1986) and references therein). However, fast growth in the model single time step is unlikely. Since a short time step is necessary to represent correct variation of friction velocity and other environmental parameters, the authors have introduced a calibration constant fine-tuned by the optimization procedure to adjust growth rate on such a short time-step. As for unit inconsistency please see comment 7 below.

2) Why is only gravitational settling considered as removal mechanism? Other dry deposition mechanisms can be relevant for particles of the assumed size (3.3 μm). How sensitive are the calculated dry deposition fluxes to assumptions on the particle diameter? The title promises new insights into microbial fluxes, but I do not see them in the abstract or conclusions. What are for instance the ‘underlying driving forces (P12,L25)’ of microbial emissions? What new insights has the combination of the flux measurements and the emission model yielded into these driving forces? Could you highlight these findings in the abstract and conclusion?

Please see reply to reviewer #1 comment 7 regarding the implementation of a deposition model considering impaction and interception.

About the 3.3 microns diameter, we chose it following Raisi et al. (2013) since, even if related to a suburban area, it was one of the most recent works where a long term monitoring of bacterial and fungal species was carried out along with aerodynamic considerations. For sizes close to the chosen diameter the average deposition fluxes are quite similar to what is presented in our paper (for a doubling of the diameter, the average difference in net fluxes is $< 10\%$ on the calibration dataset).

About the “new insights”, we aimed to highlight that microbial emissions are not driven only by turbulence, but are a more complex interaction of population dynamics, surface dynamics and atmospheric conditions, as shown with the PLaNET model. We can understand, though, that these are

not really “new” insights but rather a confirmation of what was already hypothesized in past works. Following the reviewer’s suggestion, title has been modified in “Measurements and modelling of surface-atmosphere exchange of microorganisms in Mediterranean grassland” (P1 L1-5).

3) I would like to see some more discussion on which types of microorganisms are sampled. The MS mentions viable microorganisms. Does that include both bacteria and fungal spores? Besides, can you say something about the size range of the observed particles? This will be important to eventually evaluate the role of the emitted bioaerosols on climate.

The chosen sampling medium was non-selective, thus allowing the growth of both bacteria and fungal spores and this will be clarified in the new text. Burkard samplers used in this experiment have proven to be able to sample both aerosolized *P. syringae* (see reviewer #1 comment 12) and mildew spores (Schwarzbach, 1979). Reasonably, particle size ranged from few (≈ 3) up to tens of microns (≈ 40).

4) P2,L21: in addition to these papers, (Crawford et al., 2014) measured PBA fluxes using the flux-gradient method, and (Ahlm et al., 2010; Whitehead et al., 2010) measured fluxes of coarse aerosol in tropical forests (presumably PBAs) using eddy-covariance

We thank the reviewer for these further references that have been added to the revised version of the text (P2 L22-25).

5) P5,L2: competition is mentioned here as a driver of the microbial dynamics, but I don’t think it is actually included in the model. Please limit this description to processes that are included in the model.

The revised version of the text clarifies that the PLAnET model represents the source term only as a temperature dependent growth function (P5 L19-20).

6) P5,L30: why is only gravitational settling included? For supermicron particles, also inertial impaction is important.

Please see reply to comment 2 and reply to reviewer #1 comment 7 regarding the implementation of a deposition model considering impaction and interception.

7) P6, eq8: I have some serious concerns regarding this equation, both as presented in the MS as in the code; this equation has microbial population size in the same units as the microbial growth and emission flux, which cannot be true. Should it read $dN/dt=rNF_n$? In that case, it would represent exponential growth of the microbial population and loss due to emission. In the code, it is implemented as $N(t)=N(t-1) + N(t-1)*r + F_n$, in which units are also inconsistent. It could be solved by multiplication of the 2nd and 3rd term on the RHS by the timestep, which would yield a discretization of the equation for exponential growth.

We thank the reviewer for highlighting some inconsistency that were indeed present in the manuscript, and were corrected as detailed below. As for the model code and its numerical outputs, we understand the reviewer’s concern that is due to a lack of clarity and lack of comments in the code, that however is correct and produced correct results.

Both emission and deposition fluxes are multiplied by the time step constant (ξ , in s, see Eq. (2) and Eq. (4)) therefore the correct dimensionality of F_e and F_d is CFU m⁻². New population at the time-step

t (Eq. (10) of the revised version) is computed as the product of a growth rate (r , which is a ratio of temperatures and, therefore, non-dimensional) times the population at the time step $t-1$ (in CFU m^{-2}) times the net flux ($F_e - F_d$) which, thanks to the multiplication by the time step in s of F_e and F_d , is also in CFU m^{-2} . Fluxes are converted back into $\text{CFU m}^{-2} \text{ s}^{-1}$ by dividing F_e , F_d and F_n by ξ when outputting the variables, thus all the plots are consistent with the displayed units as well.

To make this process more clear in the new version of the paper the time-step multiplication has been removed from equation 2 and 4 (yielding flux in $\text{CFU m}^{-2} \text{ s}^{-1}$) and added to the net flux term of equation 10 (thus correctly subtracting CFU m^{-2} from the population in CFU m^{-2}) (P7 L23-25). This streamlines the explanation of the model processes without affecting the results. Corrections have been made to Table 1 accordingly.

8) P6,L24: it is unclear what is meant here: ‘ k_{\min} , which is the point at which all process find an equilibrium’

The sentence (P7 L26-27) has been rephrased in the revised version of the paper to better understand the link with the reference of Waggoner (1973) (i.e.: the presence of a fraction of microorganisms sheltered by wind action).

9) P7, L14: can you discuss how this choice has affected your results? This number seems to be important in determining the upper and lower bounds of the modeled microbial population.

The choice was made to avoid the unrealistic scenario of having always all the plants’ leaves exposed to the atmosphere. However, it is a simple scaling of parameters ranging 1-2 orders of magnitude and, as it is possible to see from the model sensitivity analysis (Table 2), 10 % variations in k_{\min} and k_{\max} have minimal impact on model performance (values of $\varepsilon < 0.24$).

10) P9,L28-31: strictly spoken, the Burrows et al 2009a study does not discuss the effect of PBAP on precipitation, which is what this sentence seems to imply

Following the reviewer’s advice, we changed the sentence from “Previous attempts to understand the impact of PBAs on precipitation” to “Previous attempts to understand the distribution of PBAs in the atmosphere” (P10 L32-33).

11) P9,L32: I would add transport to ‘emission-deposition process’ (e.g. Wilkinson et al., 2012)

Following the reviewer’s advice, we added such processes and reference at the indicated point in the text (P11 L3-4 in the revised version of the paper).

12) P10,L28: what does it mean if the 95% confidence intervals include 0 and 1 or not?

The authors referred to the confidence interval for the slope of the regression. The fact that such intervals do not contain 0 implies that, even taking in account the uncertainties, the slope remains significantly different from zero (i.e. >0). Since the confidence intervals cross 1 we cannot exclude that by increasing the data point a better linear regression (with a slope close to the 1:1 line) could be achieved.

13) P11,L2-12: I miss a discussion here on the use of online detection of PBAs using fluorescence measurements (e.g. Gabey et al., 2010; Huffman et al., 2010) or single particle mass-spectrometry (Zawadowicz et al., 2017). These techniques measure concentrations, but could in principle be used in combination with micrometeorological techniques to measure fluxes (e.g. Crawford et al., 2014).

A brief discussion has been added in the revised version of the paper addressing the possibility of applying UV-LIF and SPMS to measuring microbial emissions (**P12 L33-34** and **P13 L1-13**).

14) P11,L18: it is unclear what is meant here: ‘it is not to underestimate the long-term importance of evaluating the viable fraction of said fluxes’. Please rephrase

The sentence implied that, from the evolutionary point of view as well as for any interest in pathogen transport and colonization of distance places it is important to understand how many viable microorganisms are leaving the surface, since that would be the fraction of the total microorganisms potentially able to reproduce, colonize and, eventually, attack new areas and hosts. Nevertheless we agree with the reviewer that the significance of the sentence was not really explicit. In the new revised version of the paper the sentence has been rephrased (**P13 L19-21**).

15) P12,L9-10: is rain rate given in mm/hour here?

That is correct. The unit has been added to the revised version of the paper (**P14 L15-16**).

16) Fig. 6: with half-hourly observations and model data available, why are only daily average fluxes given? In addition, it would be interesting to see time series of observations and model data.

The model needed to run at half-hourly time steps for resolving meteorological dynamics, but then daily averages were calculated in order to reduce the significant random uncertainty inherent to the 30 minutes' data.

Technical issues

17) P4,L15: unit is missing for z0

Unit (m) has been added to the revised version of the paper (**P4 L26**)

18) P5, eq 2: in the code, Nk_max is given as N/k_max, which seems correct to me, as it would express the population scaled by the carrying capacity, and judging by the units. Besides, the values of m1-m3 differ slightly from those in L19. What are the units of m1-m3? They cannot all be unitless (as mentioned in Table 1 and 2) when Fe is in CFU m-2 s-1.

Thank you for pointing this out. Units for m1 and m2 are in CFU m⁻² s⁻¹, while m3 is adimensional. Table 1 has been updated in the new version of the paper.

19) P6, eq 9: this equation seems to be missing an exponent ((Topt-Tmin)/(Tmax-Topt)), which is included in the code. What is the unit of r? Based on eq. 8 it should be s-1. Then also c should have this unit, and not none, as mentioned in Table 1 and 2. Please check these and other units throughout the MS.

We thank the reviewer for spotting the error, the exponent for Eq. (9) (**Eq. (11)** in the revised version of the paper) has been added to the new version of the text. As for the unit of r it is the result of a ratio of temperatures and an adimensional calibration constant (c , see **Eq.(11)** of the revised version of the paper) and is therefore adimensional. See also the answer to comment 7.

20) Miscellaneous typos and language corrections:

P9,L9: won't -> will not

P10,L23: remove 'it'

P10,L24: the Planet -> Planet

P11,L1: a scaling -> scaling

P11,L14: transmit -> transmitting

P11,L15: represents -> represent

P11,L16: the atmospheric -> atmospheric

P12,L6: acting -> act

P12, L32: which is nested -> which it is nested

P12,L14: has-> have

P12,L24: suggest adding a comma between 'precipitation and'

P12,L32: which is -> which it is

Technical adjustments were made following the reviewer's suggestions.

21) Fig. 3 and 5: Data within years are plotted as if they represent time series (with continuous lines), but this is not always the case. This makes the plots hard to interpret. Besides, time labels are placed at irregular intervals. Pls update these figures to make them easier to understand.

In the new version of the paper the figures have been reworked in order to remove lines, have clearer time labels and overall give a better presentation of the flux measurements (**Figures 3 and 6**).

22) In the code at L305: in the Cc calculation, a factor of 2 is missing in the exponent

We thank the reviewer for pointing out the mistake. The correction was applied, but the presented results are not heavily influenced by said mistake. With the introduction of the new impaction/interception model the settling velocity alone has become almost negligible. This correction has been nevertheless applied in the new version of the PLANET model that has been uploaded to the MathWorks File Exchange.

References cited in the point-to-point response

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~~Ecosystem-atmosphere exchange of microorganisms in a Mediterranean grassland : new insights into microbial flux through a combined experimental-modeling approach~~ Measurements and modelling of surface-atmosphere exchange of microorganisms in Mediterranean grassland

Commentato [FC1]: R2-C2

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Abstract. Microbial aerosols (mainly composed by bacterial and fungal cells), may constitute up to 74 % of the total aerosol volume. These biological aerosols are relevant not only from the point of view of the dispersion of pathogenic ~~species~~, but also ~~has due to the potential~~ geochemical implications. Some bacteria and fungi may, in fact, serve as cloud condensation or ice nuclei, potentially affecting cloud formation and precipitation and are active at higher temperatures compared to their ~~much more intensively studied~~, inorganic counterparts. Simulations of the impact of microbial aerosols on climate are still hindered by the lack of information regarding their emissions from ground sources. This present work tackles this knowledge gap by i) applying a rigorous micrometeorological approach to the estimation of microbial net fluxes above a Mediterranean grassland and ii) developing a deterministic model (the PLAnET model) to estimate these emissions on the basis of a few ~~easily recovered~~ meteorological parameters that are easy to obtain (the PLAnET model). The grassland ~~itself~~ is characterized by an abundance of positive net microbial fluxes and the model proves to be a promising tool capable of capturing the day-to-day variability in microbial fluxes with a relatively small bias and sufficient accuracy. PLAnET is still in its infancy and will benefit from future campaigns extending the available training dataset as well as the inclusion of ever more complex and critical phenomena ~~affecting-triggering~~ the release emission of microbial aerosol (such as rainfall). The model itself is also adaptable as an emission module for dispersion and chemical transport models, allowing ~~to~~ further exploration of the impact of land-cover driven microbial aerosols on the atmosphere and climate.

1 Introduction

Vegetated land surfaces, and plant leaves in particular, harbor a large number of microorganisms that can be transported by wind. It has been estimated that the planetary phyllosphere harbors about 10^{24} to 10^{26} bacterial cells (Morris et al., 2002) of the 10^{30} that live on Earth (Whitman et al., 1998). Up to 10^7 bacteria per cm^2 are present on leaf surfaces (Morris et al., 2004), and plant materials are considered the largest source of fungal spores in the atmosphere (Burge, 2002). All of these organisms can be transported into the atmosphere by wind (Delort et al., 2010), as was shown experimentally in an artificial wind-gust chamber (Lighthart et al., 1993). Atmospheric transport can involve both multiple short-distance events (Brown and Hovmøller, 2002), as well as single long-range movements. The latter are well known to transport desert dust (Rosselli et al., 2015; Peter et al., 2014; Kellogg and Griffin, 2006; Griffin, 2007), while long-range transport of epiphytic organisms living in plant canopies is much less documented. Nevertheless living and dead microorganisms are part of primary biological aerosols (PBAs) that contribute 13 to 74 % of the entire aerosol volume globally (Graham et al., 2003). Furthermore, microorganisms can be found in cloud water droplets. Water sampled from clouds over alpine regions in France and Austria contained about $2 \times 10^4 \text{ mL}^{-1}$ of bacteria (Amato et al., 2007; Bauer et al., 2003), while fungi were at least an order of magnitude lower. Different bacterial species were also found in fog droplets of the Po plain in Italy (Fuzzi et al., 1997), as well as in clouds over Scotland (Ahern et al., 2007).

The presence of microorganisms in the atmosphere may be relevant to climate processes given that some of these microorganisms can serve as cloud condensation or ice nuclei (Möhler et al., 2007; Morris et al., 2004; Szyrmer and Zawadzki, 1997; Hoose et al., 2010), potentially affecting cloud formation and climate (Amato et al., 2007). Some microbial species, in fact, are able to freeze water at temperatures significantly warmer than those induced by non-biological ice nucleators (-2 to -7 °C versus <-10 °C or -15 °C) (Morris et al., 2004). In the past, only few attempts have been made to directly measure or quantify the flux of bacteriamicroorganisms from plant canopies (Lindemann et al., 1982; Lindemann and Upper, 1985; Lighthart and Shaffer, 1994; Crawford et al., 2014) covering only some periods and some land uses. Direct eddy covariance measurements of aerosols exchange in tropical forests, where PBAs represent a significant fraction of the airborne particulate matter (Graham et al., 2003), were also performed by (Ahlm et al., 2010) and (Whitehead et al., 2010), potentially giving a proxy for microbial emission in tropical ecosystems. The mass of PBAs that is actually released by different land use types under different conditions and, more importantly, the specific composition of such fluxes and their quantification remains so far mostly unresolved. As a consequence, numerical quantification of microbial emissions, as well as investigations of the effects of living biological particles on the atmosphere and the water cycle have been limited to highly idealized scenarios. Lighthart and Kirilenko (1998) attempted to simulate summertime diurnal emission dynamics, but in their work net upward fluxes were a function of time and solar radiation-dependent microbial death only. Population dynamics in the phyllosphere and atmospheric turbulence were not accounted for. In their attempt to simulate global impacts of microbial particles on the water cycle, Hoose et al. (2010) and Sesartic et al. (2012) used fixed values of bacterial emission fluxes from different ecosystems, while Heald and Spracklen (2009) used mannitol as a proxy to evaluate fungal contributions to PBAs. Burrows et

Commentato [FC2]: R2 -C4

Commentato [FC3]: R1-C1

Commentato [FC4]: R2-C4

al. (2009a) modeled global emissions using data about airborne concentrations of microbes reported in the literature and derived fluxes from such proxies. ~~But while Burrows et al. (2009a) tried to account for time and ecosystem type in their estimates, in reality the emission fluxes are much more variable in space, time and species composition, especially because of variation in vegetation across space.~~

Commentato [FC5]: R1-C2

5 The aim of this paper is twofold: i) to increase knowledge of microbial emissions by quantifying fluxes by means of a more complex micrometeorological method compared to earlier attempts and ii) to propose a deterministic model to estimate both on-ground population dynamics and ~~the associated~~ atmospheric exchange processes.

The latter model was calibrated with microbial flux measurements made episodically over 3 years (between 2008 and 2010), while a second measurement campaign (2015) was used to validate its performance.

10 2 Materials and Methods

2.1 Flux Measurements

Microbial fluxes were measured during two field campaigns in a pasture in Montfavet, France, (43.95° N, 4.88° E, 32 m a.s.l.). Measurements were made between 7 and 11 July 2015 and again between 26 September and 1 October 2015. The pasture was a typical Mediterranean grassland dominated by grasses in an area surrounded by similar ~~land use terrain~~ and with no significant orographic features. The vegetation status was different during the two campaigns: in July the grassland showed visible signs of water stress, but no more than 20 % of the leaves were chlorotic and dry. In September the grassland was instead well developed and mostly green. The mean height of the canopy was approximately 20 cm for both campaigns. ~~The field was mainly covered with clover (*Trifolium spp.*) and ryegrass (*Lolium perenne*), was not intensively grazed and was not actively managed during the measurement campaigns with no mowing or irrigation.~~

Commentato [FC6]: R1-C6

Commentato [FC7]: R1-C3

20 Profiles of wind speed, air temperature and viable aerosols were made at two heights (≈ 67 and ≈ 227 cm), while a sonic anemometer (USA-1, Metek, Elmshorn, DE; located at ≈ 300 cm) measured 3D wind components and the sonic temperature at 20 Hz frequency. In September, an open-path infra-red gas analyzer (Li-7500, LiCor, Lincoln, Nebraska, USA) along with a differential infra-red gas analyzer (Li-7000, LiCor, Lincoln, Nebraska, USA) were added to the set-up in order to measure the CO₂ and H₂O gas exchange concurrently by the eddy-covariance and the flux-gradient method (Baldocchi et al., 1988).

25 This setup (Fig. 1) allowed to assess the performance of the flux-gradient method of estimating, water vapor fluxes vs. the respective fluxes directly measured by the eddy covariance method. Viable bioaerosols were sampled with Burkard jet samplers (Burkard Manufacturing Co. Ltd., Rickmansworth, UK). The samplers operated at a flow rate of 500 l_{min}⁻¹ and particles were collected on Petri dishes containing 10 % tryptic soja agar (1.7 g tryptone, 0.3 g peptone soja, 0.25 g glucose, 0.5 g NaCl, 0.25 g K₂HPO₄, 15 g agar per L). After sampling, the dishes were incubated at 25 °C and microbial colonies were counted after 24 h of incubation and for up to 3 days. ~~Such medium was non-selective allowing the growth of both bacteria and fungi.~~

Commentato [FC8]: R3-C3

Sampling with each Petri dish lasted for 14 minutes and for every day a handling blank was incubated alongside the sampled plates.

The design of the virtual impactor followed good design practices with a direct alignment of the nozzle and the collection probe (i.e. the still air chamber) and diameter of the collection probe (0.08 m) at least 40 % larger than the nozzle diameter (Marple and Olson, 2011). Data from literature indicate a sampling efficiency ranging from 80 to 100 % for mildew spores (Schwarzbach, 1979). Given the Burkard sampler's high flowrate, sampling happens at a super-iso-mean-velocity compared with external wind speed. The sampling efficiency is therefore expected to decrease for larger particles proportionally with the ratio between external wind speed and the Burkard's sampling speed (Brockmann, 2011).

A series of 10-minute samplings with the Burkard samplers kept at the same height was done to evaluate the minimum resolvable gradient (MRG) following Eq. (1) below (Edwards et al., 2005;Fritsche et al., 2008). An ANOVA was used to verify the absence of significant difference in the number of colonies counted between the two samplers.

$$MRG = (|\overline{A - B}|) + \sigma(A - B) , \quad (1)$$

where In Eq. (1) A indicates the sequence of number of colonies in the top sampler, B in the bottom sampler and σ the standard deviation of the respective differences.-

The flux-gradient method was used to estimate microbial fluxes from concentrations measured with the Burkard samplers. This methodology has been widely used to measure atmospheric fluxes of different scalars such as hydrogen (Meredith et al., 2014), nitrates and nitrogen compounds (Beine et al., 2003;Griffith and Galle, 2000;Taylor et al., 1999), mercury (Edwards et al., 2005;Fritsche et al., 2008;Lindberg et al., 1995) and particulate matter (Bonifacio et al., 2013;Kjelgaard et al., 2004;Park et al., 2011;Sow et al., 2009). The method follows the Monin-Obukhov similarity theory (Monin and Obukhov, 1954) and, therefore, assumes that in the atmospheric surface layer the flux of a certain scalar is a function of the gradient of the scalar measured at different heights, the heights themselves (z_i), and a transport velocity which is dependent on atmospheric turbulence and stability (a more detailed description of the methodology is provided in the supplementary material).

The measurement setup was chosen to avoid sampling in the roughness sub-layer, where the scaling principles do not hold and a conservative roughness length ($z_0 = 0.15 \text{ m}$) was chosen (Businger, 1986). This length was adequate to obtain a $z/z_0 > 1$ (Businger, 1986) at both the low and high sampling heights, thus offsetting the presence of upwind obstacles that were within the range of the required horizontal surface uniformity (≈ 25 times z meters, Irvine et al. (1997)). Coherently with the cited literature, fluxes are reported from the perspective of the atmosphere as positive when upward (i.e.: emissions) and negative when downward (i.e.: sinks).

A similar setup was employed between 2008 and 2010 to measure PBAs in an area very close by (43.91° N , 4.87° E and roughly 30 m a.s.l.). The Burkard samplers were deployed in a gradient configuration (at 50 and 250 cm above ground) along with a nearby sonic-anemometer stationed at roughly 230 cm above ground. No trace gases measurements were made during this period. The experimental field for these previous campaigns was covered with herbaceous species with similar habitus

Commentato [FC9]: R1 - C4

Commentato [FC10]: R3-C17

Commentato [FC11]: R2-C5

with similar herbaceous species such as cocksfoot (*Dactylis glomerata*), ryegrass, tall fescue (*Festuca arundinacea*) and alfalfa (*Medicago sativa*). In the 2008-2010 campaigns a different methodology was used to assess the sampling differences between the two Burkard samplers. The two samplers were put together and a serial dilution of *P. syringae* was aerosolized. Three replicate samples were taken per each dilution (10^2 , 10^3 and 10^4 live bacteria per ml) and no statistical differences were detected in the CFUs sampled by the Burkard samplers, with the single exception of one replicate at the 10^3 dilution. All the tests were conducted with an open petri dish used to verify the deposition of the aerosolized spray.

Commentato [FC12]: R1-C12

2.2 The Plant-Atmosphere Epiphytic Transport (PLAnET) model

The model estimates microbial fluxes from the phyllosphere via a set of meteorological variables (air temperature, and friction velocity and/or wind speed), the leaf area index (LAI) and atmospheric pressure. The model assumes that soil is an insignificant source of microorganisms for the atmosphere compared to the plant canopy. Other studies have considered that plant materials are the largest source of fungal spores in the atmosphere (Burge, 2002) and have shown that bacterial fluxes are higher over plants, except in cases of relatively rare events such as dust storms (Lindemann et al., 1982; Lindemann and Upper, 1985). This is in agreement with the finding that higher wind speeds are necessary to free a particle from soil rather than from the plant canopy (Jones and Harrison, 2004).

The model is based on three fundamental modules:

1. **Source:** Microbial population dynamics are driven by temperature, humidity, immigration/emigration phenomena, competition (both between the microbial species and between plants and pathogens) and these factors change throughout space and time. To reduce the complexity of such interactions, the PLAnET model limits the representation of the microbial source to its main driver as a temperature-dependent growth function.

Commentato [FC13]: R3-C5

2. **Removal:** this is an energy driven processes. Wind shear and buoyancy act on the microbial population making a fraction of it airborne.

3. **Deposition:** when employing a gradient method, what is actually measured is a net flux, which is the combination of a certain amount of a scalar becoming airborne and a certain fraction settling down. To compute the actual microbial flux, both components need to be modeled. Microbial deposition is computed as the product of a settling velocity and an airborne concentration estimated on LAI. The settling velocity itself is a linear combination of gravitational settling (computed following Kulkarni et al. (2011)) and impaction/interception (computed following Slinn (1982)).

Commentato [FC14]: R1-C7, R3-C2, R3-C6

The gross upward flux of microbes into the atmosphere was simulated following a logistic equation (Eq. (2)), assuming the existence of a threshold friction velocity (Aylor et al., 1981; Geagea et al., 1997). When simulating dust emissions it is generally assumed that there is a linear (Raupach and Lu, 2004) or exponential (Gillette and Passi, 1988) relationship between upward dust flux and friction velocity (u_*), due to the existence of saltation bombardment (Raupach and Lu, 2004; Dupont et al., 2013). Phyllosphere microbial populations are far from being comparable to the soil surface on which such bombardment occurs and, therefore, a different mechanism has been chosen in the present context. It is assumed that no saltation mechanisms can

intervene in amplifying particle removal and, therefore, the upward flux will saturate at a certain u^* . The idea for this representation of the upward flux is summed up in Fig. 2.

$$F_e = \left\{ \left[m_1 \exp(-m_2 \exp(-m_3 u^*)) \right] \xi \right\} \frac{N}{k_{max}} N k_{max}, \quad (2)$$

In Eq. (2) F_e is the gross upward flux removal (i.e.: gross upward flux (in CFU $m^{-2} s^{-1}$); m_1 , m_2 and m_3 are respectively 23.3 30 CFU $m^{-2} s^{-1}$, 255 256.26 CFU $m^{-2} s^{-1}$ and 22.3 19 and were derived through a curve fitting to the Lighthart and Shaffer (1994) flux data with FOOTPRINT92 u^* data (Lighthart and Shaffer, 1994) and the model calibration procedure; ξ is equal to 1800 seconds; N is the phyllosphere population in the model (in CFU m^{-2}), and k_{max} is the maximum allowed microbial population (“carrying capacity” in CFU m^{-2}).

If only wind speed, instead of wind speed and friction velocity, is provided as an input, the model calculates u^* using Eq. (3):

$$u^* = k u \ln \left(\frac{z}{z_0} \right), \quad (3)$$

In Eq. (3) $k=0.4$ and is the Von Karman constant; u is the wind speed (in $m s^{-1}$); z the sampling height in meters and z_0 the roughness length (=0.15 m).

The gross downward flux (i.e.: deposition) is modeled following Eq. (4) on the basis of the ratio between settling (V_g) and friction velocity since depositional effects become significant only when $V_g/u_* > 0.1$ (Gillette et al., 1974; Gillette et al., 1997).

$$F_d = (V_g + V_i) C_a \xi \begin{cases} 0 & \text{if } \frac{V_g}{u_*} \leq 0.1 \\ \frac{V_g C_a \xi \text{ if } \frac{V_g}{u_*} > 0.1}{V_g C_a \xi \text{ if } \frac{V_g}{u_*} > 0.1} & \end{cases}, \quad (4)$$

F_d is the deposition flux (CFU $m^{-2} s^{-1}$), V_g the gravitational settling velocity ($m s^{-1}$), V_i ($m s^{-1}$) the settling velocity due to impaction/interception from roughness elements and C_a the airborne concentrations of microorganisms (CFU m^{-3}).

V_g is calculated following (Kulkarni et al., 2011) (Eq. 5):

$$V_g = \frac{g \rho_p d^2 C_c}{18 \eta}, \quad (5)$$

where g is the gravitational acceleration ($9.81 m s^{-2}$); ρ_p is the particle density ($1100 Kg m^{-3}$, Cox and Wathes (1995)); d is the particle diameter ($3.3 \times 10^{-6} m$, Raisi et al. (2013) and Schlesinger et al. (2006)); C_c is the Cunningham slip correction factor; and η is the air viscosity ($1.83 \times 10^{-5} Pa s$, Kulkarni et al. (2011)).

The term V_i represents the effect of interception/impaction on particle deposition and it has been computed following Slinn (1982)

$$V_i = C_d u_r \left(1 + \frac{u_h}{u_r} \frac{1-\epsilon}{\epsilon + \sqrt{\epsilon} \tanh \gamma \sqrt{\epsilon}} \right)^{-1}, \quad (6)$$

where C_d is the ratio between u_*^2 and u_r^2 ; u_r represents wind speed measured at a reference height (in $m s^{-1}$); u_h wind speed measured at canopy height (in $m s^{-1}$) and ϵ the particle/canopy-element collection efficiency (adimensional). The latter has been computed following Slinn (1982), but without accounting for diffusional effects (E_β in the cited paper) since they are not significant for particles $> 1 \mu m$ (Wiman and Ågren, 1985). Eq. (6) has the form of a velocity (being essentially a scaling factor for wind speed) and, when combined with V_g (see Eq. (4) and (5)) determines the actual particle deposition velocity. To solve

Commentato [FC15]: R1-C8

Commentato [FC16]: R1-C7, R3-C6, R3-C6

Eq.(6) two wind speeds are needed (measured at canopy height and at a reference height above canopy), -whose ratio can be expressed as:

$$\frac{u_h}{u_r} = \frac{u_*}{ku_r} \ln \frac{l}{z_0} \quad (7)$$

where l is a characteristic eddy size in the canopy (expressed in m), which, in the present simplified implemented version of the Slinn model, has been considered equal to canopy height (h, Slinn (1982)). Following Slinn's work-paper, the parameter γ has been assumed to equal $h^{1/2}$, while the other constants were set as: $c_d/c_{d0} = 1/3$, $\bar{A} = 10 \mu\text{m}$, $\hat{A} = 1 \text{ mm}$, $F = 1\%$, $b = 2$, $c_{s0} = 1$.

The term C_a has been calculated as a characteristic seasonal airborne concentration for a Mediterranean grassland via a linear relationship between LAI values and average concentrations between the top and bottom sampler during the 2008-2010 campaign following Eq. (86):

$$C_a = p_1 \text{LAI} + p_2 \quad (86)$$

In Eq. (86) $p_1 = 26.99 \text{ CFU m}^{-3}$ and $p_2 = 115.9 \text{ CFU m}^{-3}$. LAI values used in this study were obtained from MODIS data (Myneni et al., 2015). Four 500 m pixels were averaged in space and interpolated in time to the half hourly time series from the 4-day LAI time-step of the satellite data. The average between-pixel standard deviation was quite consistent, varying slightly between ± 0.32 and ± 0.35 across all the simulated years.

The actual net PBAPs flux at a given time ($F_n, \text{CFU m}^{-2} \text{ s}^{-1}$) is computed following Eq. (79):

$$F_n = F_e - F_d \quad (79)$$

The phyllosphere microbial population (N , see Eq. (2)) is modeled following Eq. (810):

$$N = rN - (F_n \xi) \quad (810)$$

The growth rate r of Eq. (810) is modeled as a temperature-driven process in Eq. (911) (Yan and Hunt, 1999; Yin et al., 1995; Magarey et al., 2005):

$$r = \begin{cases} 0 & \text{if } T < T_{MIN} \\ \left(\frac{T_{MAX} - T}{T_{MAX} - T_{OPT}} \right) \left(\frac{T - T_{MIN}}{T_{OPT} - T_{MIN}} \right) \left(\frac{T_{OPT} - T_{MIN}}{T_{MAX} - T_{OPT}} \right) \left(\frac{T_{MAX} - T}{T_{MAX} - T_{OPT}} \right) \left(\frac{T - T_{MIN}}{T_{OPT} - T_{MIN}} \right) c & \text{if } T_{MIN} \leq T \leq T_{MAX} \\ 0 & \text{if } T > T_{MAX} \end{cases} \quad (911)$$

In Eq. (9) T_{MIN} , T_{MAX} and T_{OPT} are, respectively, the minimum, maximum and optimal growth temperatures (in °C). c is a calibration constant accounting for the unknown doubling time of the microbes in the phyllosphere. For the purpose of Eq. (10) the net flux is multiplied by the model time step ($\Delta t = 1800 \text{ s}$) making the units of the second right hand term coherent with the units of the first right hand term ($rN, \text{CFU m}^{-2}$).

The model also includes two thresholds: a minimum (k_{min} , a number of microorganisms sheltered by wind action following the concept of which is the point at which all the processes find an equilibrium, Waggoner (1973)) and a maximum population size (k_{max} or "carrying capacity" which is the maximum population that an ecosystem can sustain indefinitely, Verhulst (1838)). When the population falls below k_{min} , no removal can happen and if the population overshoots k_{max} , no growth can happen.

Since the model is focused on phyllosphere dynamics, k_{max} is appropriately scaled with LAI in order to represent plant senescence and, therefore, the reduced availability of space and resources. The model starts from an estimate of the initial

Commentato [FC17]: R1-C7, R3-C6, R3-C6

Commentato [FC18]: R3-C7

Commentato [FC19]: R3-C8

population (N_0), representing the “boundary condition” for the modeled processes at the start of the simulation and proceeds with half-hourly time-steps until the end of the simulation period.

2.3 Model Calibration and Sensitivity Analysis

5 The model was run between 1 January 2008 and the 31 December 2010, assuming that the microbial population in the phyllosphere at the beginning of the period was equal to k_{min} due to low LAI and temperature. The error metric to evaluate model performance is described by Eq. (129):

$$\varepsilon = |(1 - |s|)| + |o| + |(1 - |r^2|)|, \quad (129)$$

10 In Eq. (129), ε represents the error metric, s the slope of the linear relationship between measured and modeled net fluxes, o the offset of this relationship and r^2 is the correlation coefficient. The function receiving the model parameters as input and returning ε as an output was passed to MATLAB’s *fmincon* interior-point algorithm (Byrd et al., 1999; Byrd et al., 2000; Waltz et al., 2006) as the objective function for minimization. The algorithm was run iteratively through MATLAB’s *GlobalSearch* function in order to avoid finding a set of parameters satisfying only a local minimum. To avoid mathematically sound, but non-realistic solutions, *GlobalSearch* was looking for minima only within a bounded parameter space. The upper and lower bounds of the parameter space are shown in Table 1. The percentage of leaf area exposed to turbulence was arbitrarily estimated to correspond to 5 % of the average leaf area density of the grassland that was set at 94 g m⁻² (Sims and Singh, 1978). The latter assumption was needed to scale the measurements in CFU g⁻¹ of Hirano and Upper (1986) and Wilson and Lindow (1994) to the units needed by the model (CFU m⁻²).

20 The calibrated model was then run on the data collected in 2015 in order to assess its performance on a dataset not used for training. Optimal temperature was not entered as a calibration parameter but was assumed to be halfway through between the T_{MIN} and T_{MAX} chosen by the optimization algorithm.

Sensitivity of the model was analyzed by computing new values of ε by varying each parameter by plus and minus 10 %. For each parameter a mean ε was computed by averaging the two errors resulting by the up and down modifications. Finally, a sensitivity metric was obtained by simply subtracting the ε obtained by the optimization procedure from the average error of each parameter.

3 Results

3.1 Field Measurements

30 During the 2015 campaigns temperature ranged between 13.4 and 34.1 °C (mean 25.2 ± 6 °C, right y-axis Fig. 3a). Wind speed fluctuated between 0.2 and 5.3 m s⁻¹ (mean 2.1 ± 1.2 m s⁻¹, left y-axis Fig. 3b) with a general northerly wind direction (right y-axis Fig. 3b). During the same campaign fungal colonies dominated the microbial colonies growing on culture media, but

bacterial-like colonies were also present. Measured microbial fluxes varied both between and within the days of the two field campaigns (July and September, left y-axis Fig. 3a) with individual flux measurements being above the MRG in 60.6 % of all cases. Unreliable fluxes were unevenly distributed between July and September and included all negative fluxes (i.e., deposition, left y-axis Fig. 3a). In 2015, the plant canopy was a net microbial emitter (left y-axis Fig. 3a), with net fluxes

5 ranging between 0.2 and 28.5 CFU m⁻² s⁻¹. ~~An overview of the relationship between counted CFUs, MRG and estimated fluxes is presented in Fig. 4.~~ In September 2015, fluxes of water vapor directly measured by eddy-covariance were compared with the ones resulting from the application of the flux-gradient method, yielding a high correlation between the two ($r^2 = 0.702$) and with minimal bias ($y = 1.05x - 0.408$; RMSE = 0.798) (Fig. 54), thus ~~showing the absence of divergences between the two methods, supporting the reliability of the flux-gradient method~~

Commentato [FC20]: R1-C5b

10 The measurements ~~made between 2008 and 2010, were made in different seasons instead, spanned a wider seasonal window (with few measurements done even in February 2008)~~, resulting in a wider range of temperatures spanning from 7.9 to 28.1 °C (mean 18.5 ± 4.8 °C, right y-axis Fig. 65a). Wind speed, ~~instead, showed a high was~~ consistent with the 2015 campaign, ranging ~~between from 0.4 and to 5.8 m s⁻¹~~ (mean 2.4 ± 1.4 m s⁻¹, left y-axis Fig. 65b) ~~and, again,~~ with a mainly northerly wind direction (with the exception of 2009 for which no wind direction data were available, right y-axis Fig. 65b). Microbial fluxes

15 within these three years spanned a wider range of magnitude, varying between -5.2 and 57.1 CFU m⁻² s⁻¹ (left y-axis Fig. 65a). The average flux between 2008 and 2010 was close to the 2015 average (8.3 CFU m⁻² s⁻¹ in 2008-2010 versus 10.6 CFU m⁻² s⁻¹ in 2015), ~~while the standard deviation was higher but between 2008 and 2010 fluxes showed a higher spread around the mean (a standard deviation of 11.1 CFU m⁻² s⁻¹ in 2008-2010 versus 6.2 CFU m⁻² s⁻¹ in 2015).~~ ~~Few Even if some~~ negative fluxes were registered in 2008-2010, ~~these were a minority compared to the positive ones. Indeed, negative fluxes that~~ represented

20 only 16.8 % of ~~the total all the fluxes measured during this period~~, confirming that the sampling site tended to be a net microbial emitter, rather than a sink.

Commentato [FC21]: R1-C11

3.2 Model Calibration

The results of the optimization ~~ares~~ resumed in Table 2 where the chosen parameters are reported along with the respective sensitivity value.

25 All the chosen values fell within the imposed boundaries (see Table 1), ~~with only one parameter (m_3) falling on the edge of the boundaries~~. The optimization procedure was able to find a meaningful optimum as it is deducible by looking at the sensitivity values reported in Table 2. Any variation of a parameter results in a worsening of the error metric (i.e.: a positive sensitivity value), even if the model is not equally sensitive to all the parameters. More specifically, the ~~minimum temperature temperature values~~ regulating the growth curve of the microorganisms ~~is the one are those~~ with the highest impact on model

30 performance, while the minimum population size (k_{min}) seems to ~~have the least impact be less sensitive~~. The latter result also suggests that the approximation made concerning the percent of leaf area exposed to turbulence (i.e. 5 %) is not critical.

Relationships between measured and modeled fluxes with an optimal set of parameters are reported in Fig. 76 for both the calibration set (2008-2010, Fig. 76a) and the validation campaigns (2015, Fig. 76b). The model is consistent between the two

campaigns, explaining roughly ~~55-70~~60 % of the variance (r^2 for 2008-2010 is ~~0.59-54~~ while r^2 for 2015 is ~~0.57~~68) and it does it with a ~~small~~minimal offset (~~0.88~~0.28 in 2008-2010 and ~~0.4~~3.75 CFU m⁻² s⁻¹ for 2015). The model still has a bias in the flux estimation: it tends to underestimate the fluxes during the calibration campaign (slope of the regression of ~~0.71~~70) and ~~slightly~~ overestimate them during the 2015 field campaigns (slope of the regression ~~1.05~~31). The model has an RMSE of ~~5.22-5.82~~

5 CFU m⁻² s⁻¹ in 2008-2010, while it is ~~2.64~~2.78 for 2015.

Interestingly, while a clear dependence of the measured fluxes on atmospheric turbulence (u^*) was frequently observed, u^* was not always correlated with flux contrary to what might be expected. On some occasions, the measured microbial fluxes were much lower than predicted by Eq. (2) which directly scales the effect of turbulence on the microbial fluxes. This observation is consistent with the assumptions made in the PLAnET model, where the actual microbial flux is indeed driven by turbulence, but also constrained by the rate at which microorganisms multiply and by the size of the microbial population in the phyllosphere. This is represented graphically in Fig. ~~87a~~ and ~~87b~~. When the population ~~is close to falls below~~ the minimum population (k_{min}), even very high turbulence (mean u^* for Fig. ~~87a~~ is 0.49 m s⁻¹) ~~will not won't~~ elicit significant upward net fluxes. Conversely, when the microbial population is large (in Fig. ~~87b~~ above ~~4.83~~ x 10⁵ CFU m⁻²), even low turbulence (mean u^* for Fig. ~~87b~~ is 0.39 m s⁻¹) will generate significant upward net fluxes. Accordingly, the model was able to capture the variability in the amount of CFUs that can be instantaneously transported into the atmosphere, thus predicting complex interactions between weather conditions, microbial population densities and the actual flux. This latter result suggests that for organic particles the simple knowledge of the transport field may not be enough: microbial populations have their own inherent dynamics (growth, death, immigration and emigration) that influence the amount of microbial cells available for transport. This phenomenon is clearly absent in the modelling of dispersion of inorganic dust particles.

10 Simulated daily sums of PBA fluxes for the entire validation year are shown in Fig. ~~98~~. These are high from Spring to early Autumn, when temperatures are favorable for microbial and plant growth and sharply decrease during Winter months in response to a decrease in temperature and LAI, an increase in the mean wind speed accompanied by a decrease in the mean number of microorganisms populating the phyllosphere (Fig. ~~98~~). The model also predicts episodes in which the daily fluxes of microorganisms into the atmosphere are above and up to roughly twice that of the seasonal average. These events are often associated with persistent conditions of high wind and turbulence (Fig. ~~109a~~, ~~109b~~) and clear skies (Fig. ~~109c~~, ~~109d~~) which are typical of the synoptic weather conditions in Southeastern France, when high pressure in the Bay of Biscay and a low around the Gulf of Genoa generate the wind that prevails from the north (called the “Mistral”). Under these favorable conditions microbial growth in the phyllosphere balances the high removal rates caused by turbulence, so that the overall microbial population on leaves sustains high transport (Fig. ~~109c~~).

30 4. Discussion

The results and tools we present here offer a new approach for studying bioaerosols. ~~Previous attempts to understand the distribution of PBAs in the atmosphere impact of PBAs on precipitation~~ tended to simplify the surface-atmosphere transport

Commentato [FC22]: R3-C10

both by deriving emissions from airborne concentrations (Burrows et al., 2009a) and by making ecosystem-wide assumptions about emissions (Burrows et al., 2009a;Hoose et al., 2010;Sesartic et al., 2012). Airborne concentrations are, nevertheless, variable, being the ~~combined results of both emissive and depositional processes as well as atmospheric transport~~ (Wilkinson et al., 2012) ~~end result of all the emission-deposition process~~. For this reason, we conceived the PLaNET model to estimate

5 fluxes directly, while accounting for the underlying emission-deposition processes. We sought to capture the dynamics underlying microbial emissions, thereby making the airborne concentrations a direct consequence without further assumptions. The model tries to generate fluxes from the interactions of the phyllosphere population dynamics and the local meteorological conditions instead of employing only a regression framework from measured data (such as in a previous attempt to simulate microbial fluxes Lighthart and Kirilenko (1998)). While both gross upward and downward flux in the PLaNET model ~~are~~
10 ~~could be~~ resolved separately, this does not happen when employing a gradient method, and the presence of ~~depositional a gravitational effects~~ could ~~affect generate a bias in~~ the observed gradient. ~~Therefore we have computed the ratio between settling and friction velocity for the observations using the same particle diameter as in the model simulations by Raisi et al. (2013). Following~~ (Gillette et al., 1974;Gillette et al., 1997), ~~depositional effect for particles < 10 μm are significant only when the ratio between the deposition and friction velocity is greater than 0.1.~~The value of this ratio did not exceed the critical
15 thresholds ~~neither in the 2008-2010 campaigns, nor in 2015,~~ ~~giving us confidence in This guarantee~~ the applicability of the gradient method for the observations at the Montfavet site, ~~made in sufficiently turbulent conditions since they happened during moments where turbulence was sufficient for generating meaningful gradients. Depositional effects were not relevant also in Park et al. (2011) when applying the gradient method to PM₁₀ fluxes. It has to be taken in account, though, that deposition depends on the particle diameter, and the choice of a fixed diameter for bioaerosols that was made here is a necessary~~
20 ~~simplification due to the impossibility of knowing the full size spectrum and its temporal variation. Seasonal variations in the size fraction containing most bioaerosols were in fact detected by Raisi et al. (2013).~~

The PLaNET deterministic framework follows the approach of Fall et al. (2016), which employed data from the literature on a specific pathogen, *Bremia lactucae*, to estimate its airborne concentrations. From Table 2 it is clear that the optimization procedure made a clear use of the imposed bounds in order to obtain feasible parameters. Not imposing feasible bounds would
25 have posed a risk for the minimization to wander into physically unrealistic, but mathematically sound parameter space (i.e.: a set of parameters achieving a very small ϵ by combining, for example, non-realistic growth temperatures). ~~The fact that only a single parameter was constraining the optimization algorithm suggests that the chosen ranges were meaningful in both physical and mathematical terms.~~ The optimal temperature chosen by the optimization algorithm for microbial growth, for example, is 21.6 °C. Considering that during summer days with higher vapor pressure deficit the leaf surface temperature can reach even a 5 °C difference from air temperature (Jackson et al., 1981;Wiegand and Namken, 1966) this would mean that the modeled optimal temperature is quite close to the incubation temperature used in the laboratory (25 °C). ~~It is worth noting that, while a reasonable choice of growth temperature range was made for the overall microbial population, specific microorganisms may have different temperature optima~~ Future work can be done to fine tune such range on the species composition of the

Commentato [FC23]: R3-C11

Commentato [FC24]: R1-C9

microbial source. The reliability of the optimization is backed up by the sensitivity analysis: any variation in the chosen parameters results in a worsening of the error statistic, as it is clearly visible from Table 2

Compared to the model by Fall et al. (2016), the PLaNET falls short in terms of validation statistics. These differences can be explained by the different endpoints and scopes between the models. Since the PLaNET model aims to simulate an overall bioaerosol flux, instead of airborne concentrations of a single species as does the model by Fall et al. (2016), there are significant higher uncertainties involved in the process. Nevertheless, if the confidence intervals (CIs) for the slopes of Fig. 76 are taken into account, it can be seen that in 2008-2010 and in 2015, the 95 % CIs include 1 and exclude 0 (the 95 % CIs are 0.36-1.05, 0.4-1.03 and 0.41-2.21, 1.3-1.97, respectively). This suggests that the main weakness of the model would only be the number of observations. Longer campaigns conducted on different ecosystems would help in better assessing the relationship between modeled and measured data as well as the “portability” of the PLaNET model to different ecosystems.

While the obtained results are quite promising, there are still some caveats to consider. One of the first improvements that would benefit the PLaNET model would be validation on microbial fluxes that are not based solely on cultivated microorganisms. The culturable to total ratio may range from 0.01 to 75 % and is generally below 10 % (see Burrows et al. (2009b) and references therein), meaning that PLaNET output needs α -scaling to be compared with the work, for example, of

Burrows et al. (2009a), Sesartic et al. (2012) and Sesartic et al. (2013). A simple comparison can be made between PLaNET simulated fluxes and fluxes reported in Burrows et al. (2009a) using the scaling factor for culturable-to-total bacteria for grasslands (302, Burrows et al. (2009b)). An average total microorganisms flux of 750.5 ± 1976 cells $m^{-2} s^{-1}$ was obtained for PLaNET that, although associated with a high variability, is similar to the median value for grassland (roughly 1000 cells $m^{-2} s^{-1}$) reported in Burrows et al. (2009a). PLaNET can be used to predict microbial emissions and, when proper scaling factors are selected, it can be potentially used as a tool to link surface processes to the spatial and temporal dynamics of atmospheric processes since PBAs could represent an important component of the atmospheric aerosol load (at least at regional scales (Hummel et al., 2015)). There are only few quantitative, field-deployable sampling methods that target all microbial cells including non culturable ones. One of the most reliable that can be adopted for analyzing samples coming from different kinds of collectors is epifluorescence staining, which is able to discriminate biological versus non-biological particles in a sample

independently from their ~~culturability~~ viability. Contrary to plate incubation, epifluorescence is able also to detect viable-but-nonculturable microbial cells, that is, organisms that are not dead, but are not in the condition of growing (see Oliver (1993) and Burrows et al. (2009b) and references therein). The key issue with the method is the need for a minimum amount of particles per sample (10^4 per liter, Gandolfi et al. (2013)) and, therefore, an appropriate amount of sampled air. On rural sites Bowers et al. (2011) were able to employ epifluorescence sampling at $30 l min^{-1}$ for 1.5 h, while Harrison et al. (2005) worked

with high-volumetric sampling at $1000 l min^{-1}$ for 6 hours at a time. Such timescales are not suitable for flux-gradient applications, where fluctuations in concentrations must be resolvable on a timescale appropriate for the PBL response time (≤ 1 h). This is why in the present work Burkardt samplers were chosen: both for their high volumetric flowrate ($500 l min^{-1}$) and for their virtual impactor nature that is favorable for preserving particle viability. Still, in future studies, epifluorescence sampling performed on “relatively long” time intervals (e.g.: 1.5 h or more) could be used alongside more frequent (e.g.: 15

minutes) sampling.

Commentato [FC25]: R2-C1

Commentato [FC26]: R2-C3

min) cultivable samplings to scale cultivable to total microorganisms, assuming that the culturability does not change in the longer time span. Ultraviolet induced laser fluorescence (UV-LIF) is a very recent methodology that measures PBAs concentration from fluorescence emission and particles' characteristics, using statistical methods such as hierarchical agglomerative cluster analysis to distinguish between different types of PBAs Crawford et al. (2015). UV-LIF has already

Commentato [FC27]: R2 – C2

5 been used to measure atmospheric PBAs (Huffman et al., 2010; Gabey et al., 2010) and, given its relatively fast response time, it has the potential to be used in combination with micrometeorological methods to estimate microbial fluxes. A first attempt in this sense has been made in a pine forest by (Crawford et al., 2014). This method is very promising since it works independently from microbial culturability, even if research is still ongoing on discriminating between different PBAs classes and between PBAs and non-biological fluorescent compounds contaminating the signal (Gabey et al., 2013; Pöhlker et al., 10 2012; Toprak and Schnaiter, 2013). Single mass particle spectrometry (SPMS) is also a technique that can be used to detect PBAs by relying on the spectroscopic detection of specific compounds that are assumed as proxy of bioaerosols (Zawadowicz et al., 2017). Similarly to UV-LIF, this method does not rely on PBAs culturability while suffering from interference of non-biological particles having coincident spectral peaks (Zawadowicz et al., 2017). It is important to consider, though, that even

Commentato [FC28]: R3 – C13

15 the latter would not matter from an evolutionary perspective. Live cells have a chance of further transmitting their biological and chemical footprints to a wider microbial population. The latter would represent an ecological feedback increasing the population of live particles with characteristics that could favor survival and, eventually, physical interaction with the atmospheric processes (i.e.: increased expression of given proteins). While it is true that estimating fluxes of total biological particles is important from a biogeochemical point of view Burrows et al. (2009a), measuring the viable fraction of fluxes

Commentato [FC29]: R3 – C14

20 would give information about the amount of microorganisms that can potentially survive transport it is not to underestimate the long-term importance of evaluating the viable fraction of said fluxes. The second critical improvement would be to validate and test the model on data from a larger number of different ecosystems, such as forests and agricultural crops. Another caveat regards the parametrization of deposition. The simplified version of the model of Slinn (1982) implemented in PLAnET does not take into account the presence of potential negative gradients between atmosphere and canopy, which

Commentato [FC30]: R1-C7

25 was not possible to investigate during the present sampling campaigns. This aspect needs further investigation for a better representation of particle deposition in such conditions.

The prognostic capability of the model has been investigated by running the model between 2001 and 2015. In the years where meteorological data from Montfavet were not available (2001-2006), we used the average of the closest four points from the Climate Forecast System Reanalysis hourly time series (Saha et al., 2010). Season-averaged net fluxes showed small inter-annual variation in winter when fluxes fluctuated around zero (-0.01 to 0.256 CFU m⁻² s⁻¹) versus higher and more variable average fluxes in summer ranging from 3.38 to 8.38 CFU m⁻² s⁻¹. The model was able to represent the interplay of the different meteorological variables: summer 2003 was characterized by one of the lowest average phyllosphere population sizes due to the exceptionally high temperatures registered during that year hindering microbial growth. In fact, in summer 2003 the average population size was 2.8574 x 10⁵ CFU m⁻² versus a seasonal average of 3.864 x 10⁵ CFU m⁻² and an average temperature

of 26°C versus 23°C. The lowest average summer population size was simulated, instead, in 2006 (~~2.6657~~ $\times 10^5$ CFU m⁻²), when an unusually low LAI (0.8 versus 0.91 inter-annual seasonal average) influenced the maximum amount of microorganisms that were able to grow along with a high average friction velocity increasing microbial removal (0.33 versus 0.25 m s⁻¹ inter-annual seasonal average). Still, there are some potentially unresolved meteorological forcings that the model does not take into account, such as rainfall. Huffman et al. (2013) and Prenni et al. (2013) provided convincing experimental evidence that bioaerosol and ice nucleator concentrations increased during and shortly after rainfall events. Rainfall, in fact, may boost PBA emissions. On the one hand this may be due to the impact of raindrops that shake plants generating a detaching force (McCartney, 1991; Robertson and Alexander, 1994) and on the other hand it may be due to a boost in growth and a subsequent increase in the concentration of PBAs on the plant. For example, for *P. syringae* a 35-fold increase was seen after 48 hours from a precipitation event (Hirano et al., 1996). This would increase the population of particles available for transport. This seems to contrast classical wet scavenging theory, where falling hydrometeors deplete the atmosphere of suspended particles (Seinfeld and Pandis, 2012), and, therefore, acting as a sink for particulate matter (see for example Tai et al. (2010) or Ouyang et al. (2015)). Due to this complex interaction between PBAs and rainfall, precipitation and humidity were not taken into account in this first version of the PLANET model. In fact, if a very rainy month (September 2010 with an average of 0.13 mm of rain per hour) is compared against a non-rainy month (September 2007 with an average rainfall of 0.027 mm hr⁻¹), average net fluxes are very similar and actually greater in the less rainy month (~~4.3555~~ $4.875.49$ CFU m⁻² s⁻¹ in September 2007 versus ~~4.3555~~ CFU m⁻² s⁻¹ in September 2010).

But rainfall is not the only process that can influence net fluxes: intensive grazing, mowing and harvesting are also activities that can impact bioaerosols emission from a grassland. Such effects, however, are not straightforward and not completely known. Intensive grazing, for example, would damage the canopy, affecting LAI and the available population. On the other hand, by damaging plants and releasing nutrients from plant tissue it could enhance microbial growth on leaves. Furthermore animals themselves are potential bioaerosols sources (animal manure contains a large variety of microorganisms Cotta et al. (2003)). Harvesting would also contribute to the reduction of the source term (reducing LAI), but, along with many agricultural operations, it can generate a higher amount of suspended particles (see for example Hiscox et al. (2008)), potentially containing bioaerosols. All of these complex interactions can therefore generate both transient and lagged effects, which are still not taken into account by any model and should be investigated in the future.

Commentato [FC31]: R3-C15

Commentato [FC32]: R3-C15

Commentato [FC33]: R1 – C13

5. Conclusions

This study investigated with multiple campaigns the behavior of a Mediterranean grassland from the point of view of microbial emissions. Across the campaigns fluxes of microorganism have been estimated through a sound micrometeorological method (flux-gradient methodology). The applicability of the method was assessed by comparing water vapor gradient fluxes -with those measured directly by eddy covariance: the lack of significant divergence between the two, suggests that the gradient methodology was applicable in the experimental conditions, even if it needs to be acknowledged that the good correspondence

~~in terms of water vapor fluxes does not necessary apply to bioaerosol flux measurements. The soundness of said method was assessed by comparing water vapor gradient fluxes with those measured directly by eddy covariance. The good match between the two (Fig. 4), provides substantial evidence that the methodology was appropriate to estimate the fluxes of any other scalar, including the release of viable microbial particles capable of forming colonies when cultivated on appropriate growth medium.~~

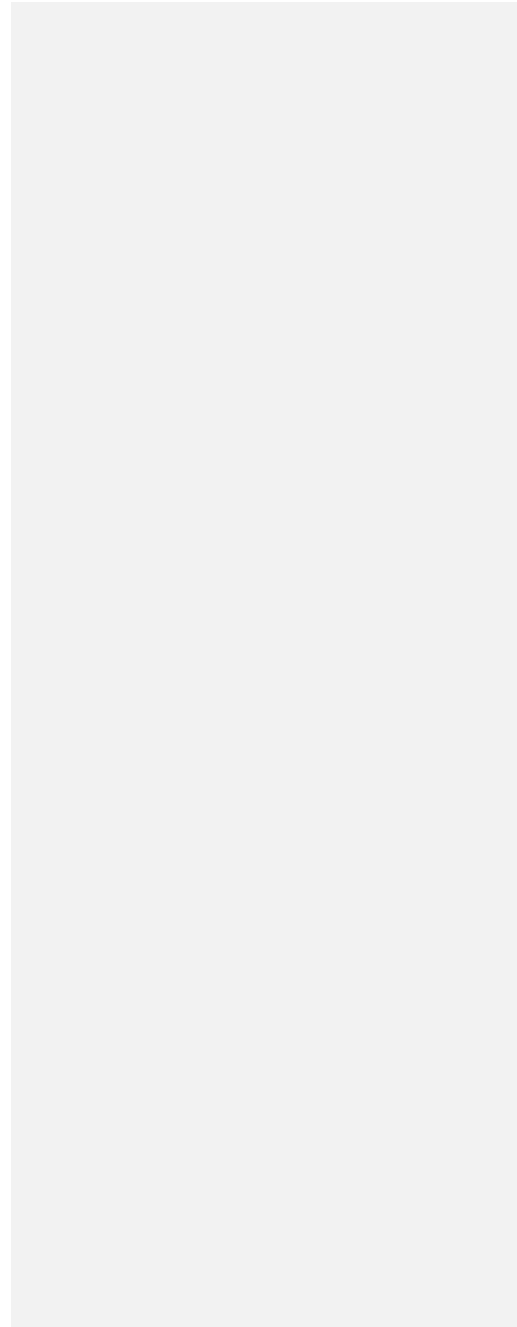
Commentato [FC34]: R1-C11

5 ~~Bacterial and fungal colonies, in fact, The latter statement is true when colonies~~ behave as a passive tracer ~~in the absence and the lack~~ of significant aerodynamic effects; ~~such condition~~ was therefore tested ~~during field campaigns for the experimental conditions~~ using literature relationships between friction velocity and size of transported particles. The grassland showed a majority of ~~emission fluxes outward fluxes~~, with a magnitude comparable to what was previously seen on a desert scrubland (Lighthart and Shaffer, 1994). The collected data were used to calibrate and validate a deterministic model (PLAnET) for
10 estimating emission of microorganisms from the surface. Even if there are still some open issues in the model (namely the relationship between flux ~~and~~ precipitation and the culturability of microorganisms), PLAnET provides previously unavailable insights into the dynamics of microbial fluxes and the underlying driving forces. Hopefully its evolution within the scientific community will be fostered not only by its ease of use (few easily accessible meteorological parameters are needed for its operation), but also by the robust framework for estimation of microbial fluxes (the model code can be freely
15 downloaded at <https://it.mathworks.com/matlabcentral/fileexchange/63257-planet-microbial-model>). Its computational simplicity makes it also an attractive addition to larger scale models aiming at simulating the dispersion of PBAs at regional or global scales (such as CALPUFF, Scire et al. (2000); WRF-CHEM, Grell et al. (2005); CHIMERE, Menut et al. (2013); ECHAM-HAM, Stier et al. (2005) -or CAMx). The PLAnET model can in fact be an emission module receiving the same meteorological forcing of the dispersion model within which ~~it~~ is nested and generate time-varying emission rates at surface
20 level. PBAs could be added as a chemical species with given characteristics, in addition to all the aerosols coming from biogenic and anthropogenic inventories. The larger-scale model would be then able to simulate their dispersion across the simulation domain giving new insight in the potential of PBAs to impact precipitation, as well as exploring different scenarios about the potential pathways of transport of plant pathogens from the phyllosphere. In fact, given that some of the aforementioned models are able to simulate both gas-phase and aerosol chemistry, it would be possible to follow up the
25 pioneering work of Burrows et al. (2009a) and Sesartic et al. (2013) at different spatiotemporal scales and investigate the changes in the outputs due to the insertion of a realistic PBAs emission module.

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Parameter	Range Boundaries	References for Ranges	Units
T_{MIN}^*	5-15	Standard Mesophilic Range	°C
T_{MAX}^*	30-45	Standard Mesophilic Range	°C
T_{OPT}	T_{MIN} - T_{MAX}	Standard Mesophilic Range	°C
c^*	0.1-2	Inserted by the authors	None
k_{min}^*	4.7×10^4 - $4.7e \times 10^5$	Hirano and Upper (1986); Wilson and Lindow (1994)	CFU m ⁻²
k_{max}^*	4.7×10^5 - 4.7×10^8	k_{min} and Hirano and Upper (1986)	CFU m ⁻²
m_1^*	22.3- 3523.3	Derived by the authors from Lighthart and Shaffer (1994)	CFU m⁻² s⁻¹ ¹ None
m_2^*	250-260	Derived by the authors from Lighthart and Shaffer (1994)	CFU m⁻² s⁻¹ ¹ None
m_3^*	1721.3 -23.3	Derived by the authors from Lighthart and Shaffer (1994)	None
ξ_d	1800	Equation (106)	Seconds
p_1	26.99	Derived by the authors	CFU m⁻³ None
p_2	115.9	Derived by the authors	CFU m⁻³ None

Table 1: Estimates for model parameters. The parameters tagged with an asterisk (*) are those that entered the calibration as unknowns from the initial guess, while the other parameters were fixed.

	T _{MIN} (°C)	T _{MAX} (°C)	c	k _{min} (CFU m ⁻²)	k _{max} (CFU m ⁻²)	m ₁	m ₂	m ₃
Value	12.96	30.16	0.13	5x10 ⁴	4.82x10 ⁶	3022.97	256.26250.06	1921.3
Sensitivity	0.654.16	0.290.40	0.230.24	0.03	0.230.34	0.350.30	0.080.02	0.470.49

Table 2: Results of the optimization procedure and the sensitivity analysis. The first row reports the value chosen by the optimization for the parameters in the column headers, while the second row reports the sensitivity value for each parameter.

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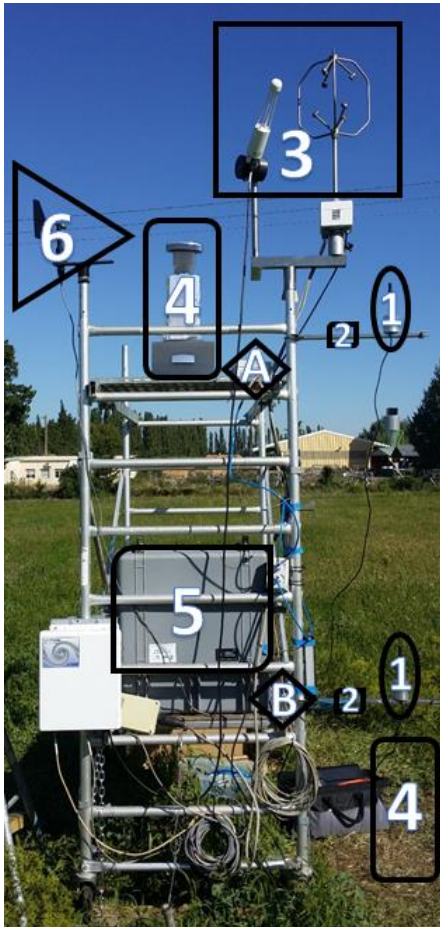


Figure 1: Schematic representation of the sampling station: each piece of equipment is represented by a number (in bold face) and the position of the equipment, expressed as cm a.g.l is indicated in parenthesis. Cup anemometers (1; 80 and 210) , thermocouples (2; 80 and 210), sonic anemometer and Li-7500 open-path gas analyzer (3; 300), Burkardt air samplers (4; 75 and 255, the bottom one is not in place in the figure, but the rectangle indicates its approximate position), Li-7000 differential gas analyzer (5; with inlets at 55 A and 200 B) and wind vane (6; 250).

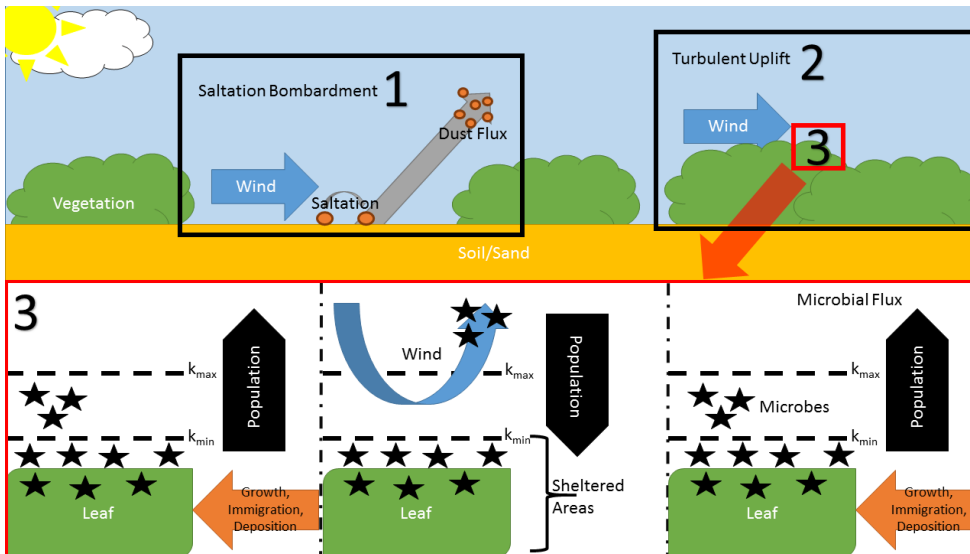
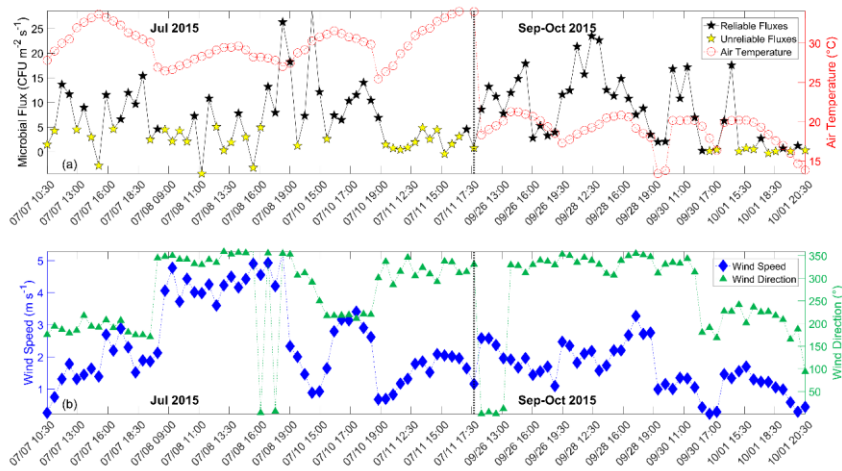


Figure 2: Schematics of the differences between Aeolian dust flux and microbial flux on which the PLaNET model is founded. Box 1 (top left) shows the typical dust saltation mechanism. The action of wind (blue arrow) on soil makes dust particles (orange dots) “jump” for short distances, ejecting smaller dust particles in the atmosphere. Turbulent uplift is shown in Box 2 (upper right) where wind acts on the phyllosphere. What happens in the small red box 3 is zoomed in into the lower part of the figure (as indicated by the red arrow). Phyllosphere harbors a given amount of microbial particles (black stars) up to a maximum (carrying capacity, k_{max} in the figure). Wind can remove a certain fraction of “available” microorganisms up to the limit of a sheltered fraction of the population (k_{min} in the figure). While the action of wind decreases the amount of particles on the leaf, the population keeps experiencing phenomena such as growth, immigration from other leaves and deposition of airborne particles, all contributing to an increase in population. The balance between population dynamics and uplift is what contribute to the net flux simulated by the PLaNET model.



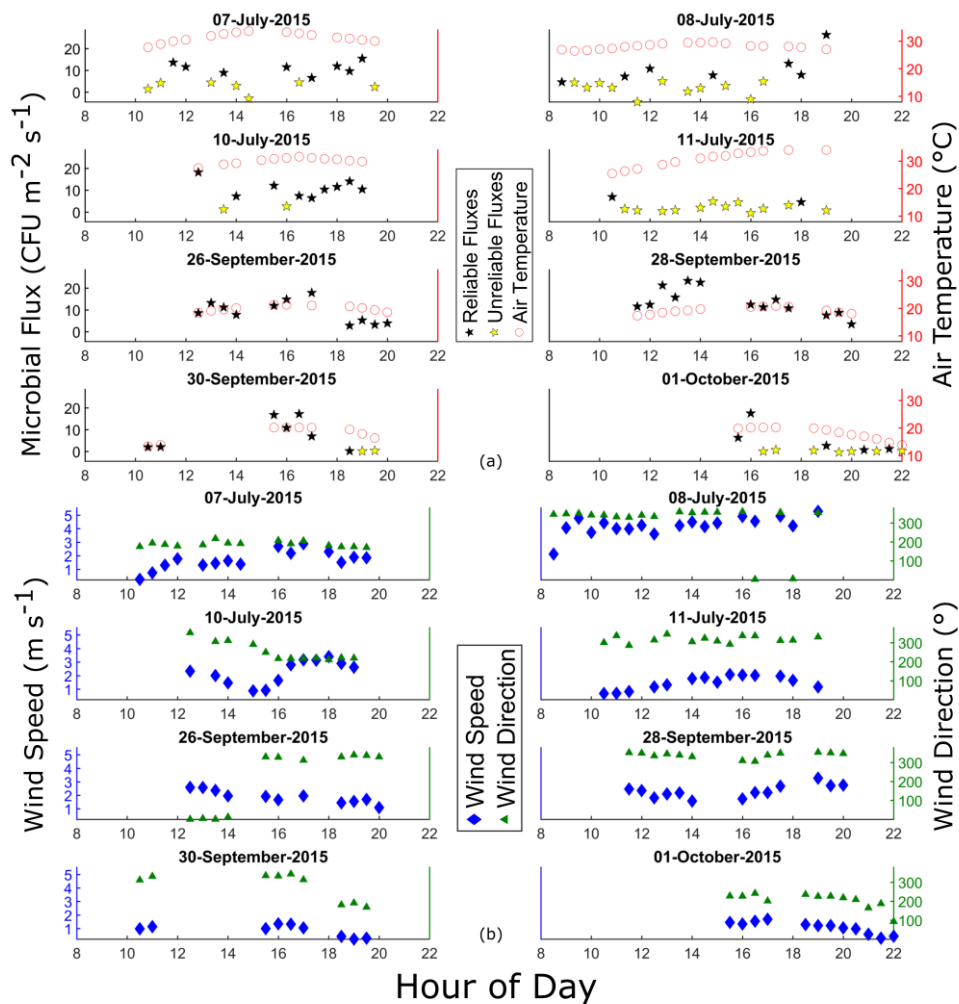


Figure 33: Dynamics of observed microbial fluxes, air temperature, wind speed and wind direction in the 2015 field campaigns. Fig. 3a shows the time series of air temperature and microbial fluxes for July and September-October 2015. Unreliable fluxes are those below the MRG. Fig. 3b shows the time series of wind speed and wind direction for the same periods. For both figure 3a and 3b dates are in the format MM-DD hh:mm, where MM indicates the month, DD the day, hh the hour and mm the minutes. To optimize space year was indicated with text inside the figure (both figure 3a and 3b refers to 2015).

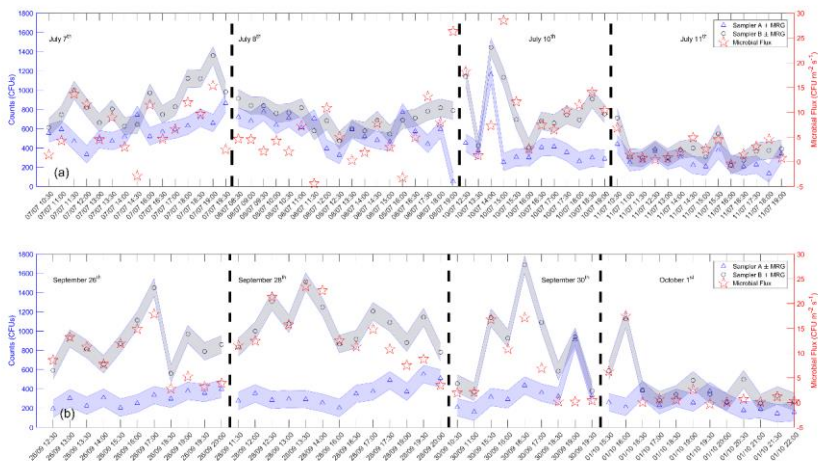


Figure 4: Details on the relationship between counted CFUs by the Burkard samplers and the estimated fluxes for the campaigns of July (a) and September (b) 2015

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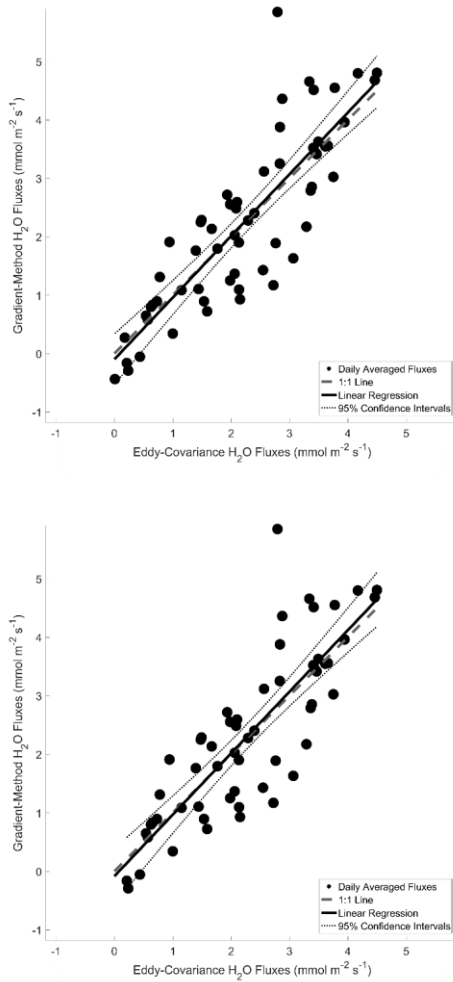
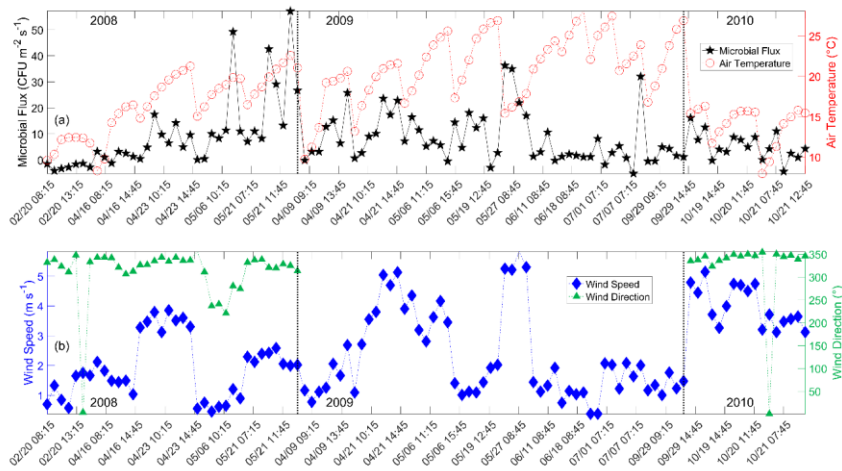


Figure 45: Water vapor fluxes measured via the eddy covariance method (x-axis) *versus* those derived from the flux-gradient method (y-axis). The plotted linear regression has a slope of 1.05, an offset of -0.089 and explains 70% of the data variability.

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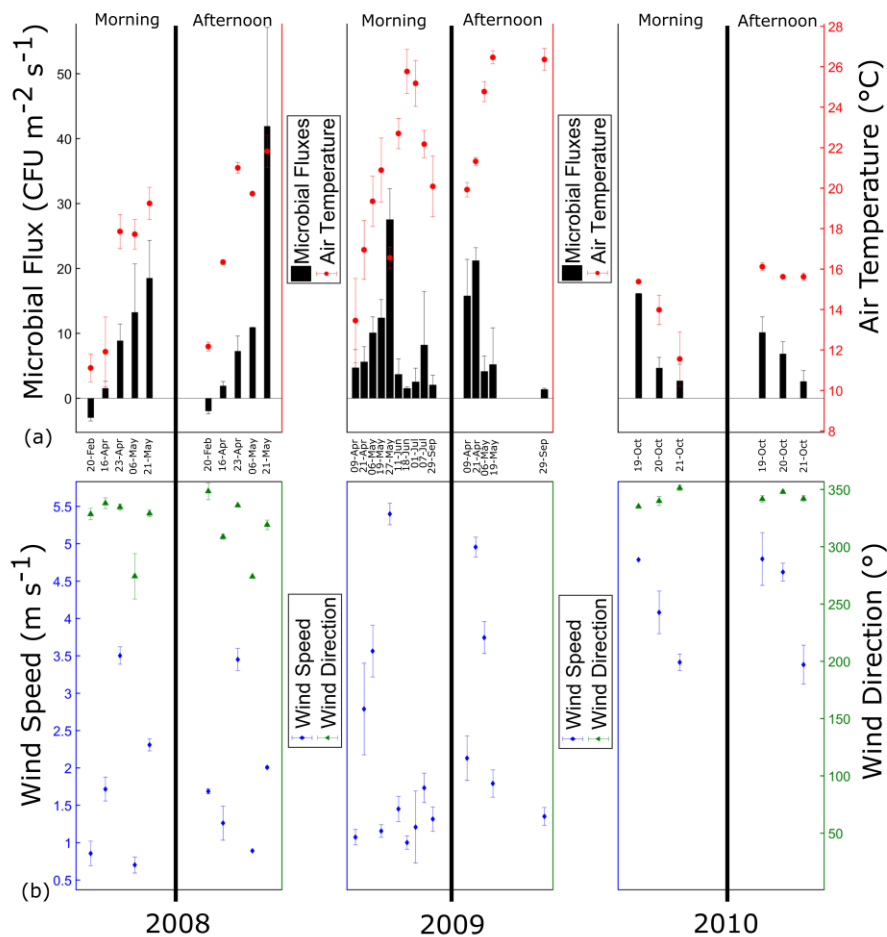


Figure 56: Temporal dynamics of observed microbial fluxes, air temperature, wind speed and wind direction in the 2008, 2009 and 2010 field campaigns. Fig. 5a-6a shows time series of air temperature and microbial fluxes for the campaigns held between 2008 and 2010. Fig. 5b-6b shows the time series of wind speed and wind direction for the same period. No wind direction data were available for the year 2009. For both figure 5a and 5b dates are in the format MM/DD hh:mm, where MM indicates the month, DD the day, hh the hour and mm the minutes. To optimize space year was instead indicated with text inside the figure, with different years separated by dashed vertical lines. For both figures 6a and 6b morning and afternoon averages are reported with the relative standard error in the error-bars.

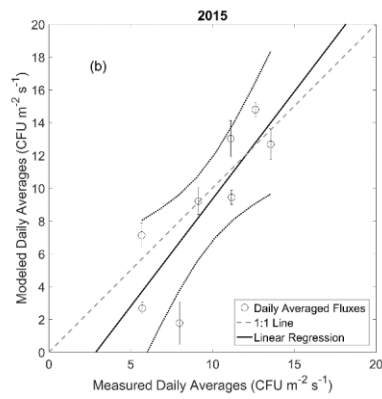
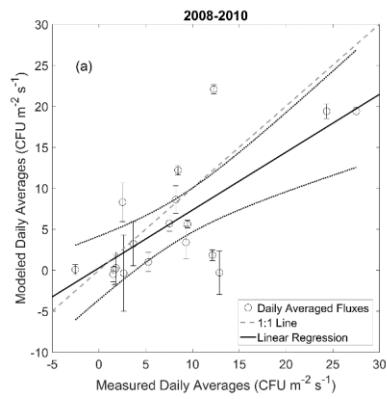
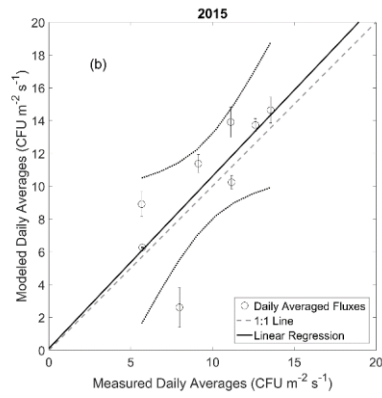
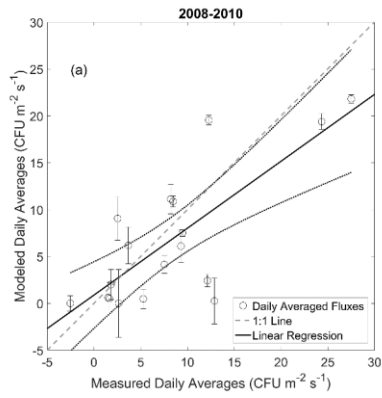
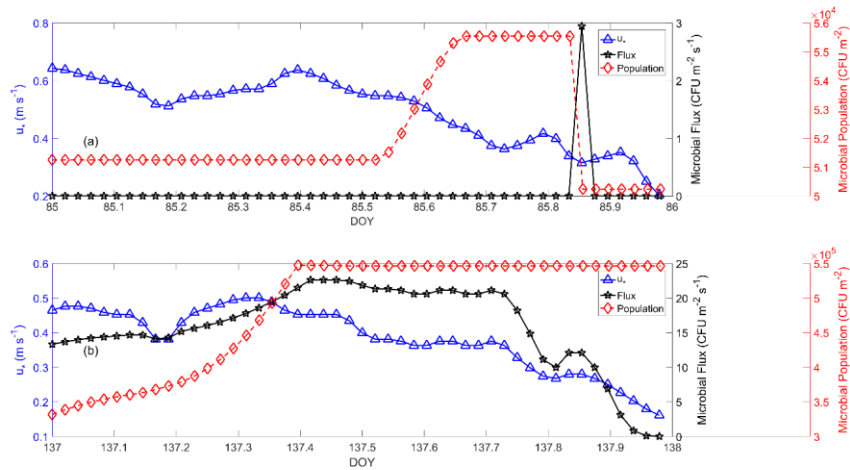


Figure 67: Relationship between measured and modeled daily averages of microbial flux. Fig. 6a shows the regression between the daily averages from the 2008-2010 campaigns and the optimized model ($y=0.70x+0.8828$; $r^2=0.5954$) and Fig. 6b shows the one from the 2015

campaigns and the model ($y=1.05 \times 31x+0.11-3.75$; $r^2=0.5768$). The error bars are derived from the following equation $\pm \sigma \left(\frac{m_i - o_i}{\overline{m_i, o_i}} \right)$. Where σ is the standard deviation of the ratio between the difference between modeled and observed points ($m_i - o_i$) and the average of the same points ($\overline{m_i, o_i}$).

Formattato: Tipo di carattere: Non Grassetto

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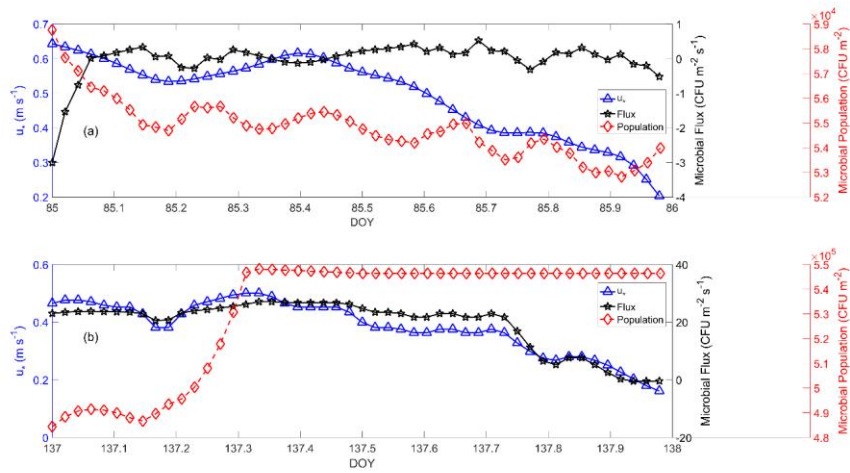
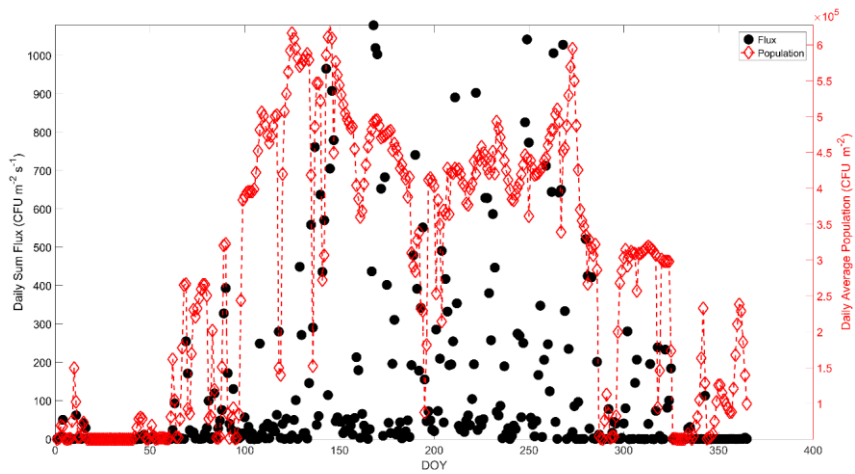
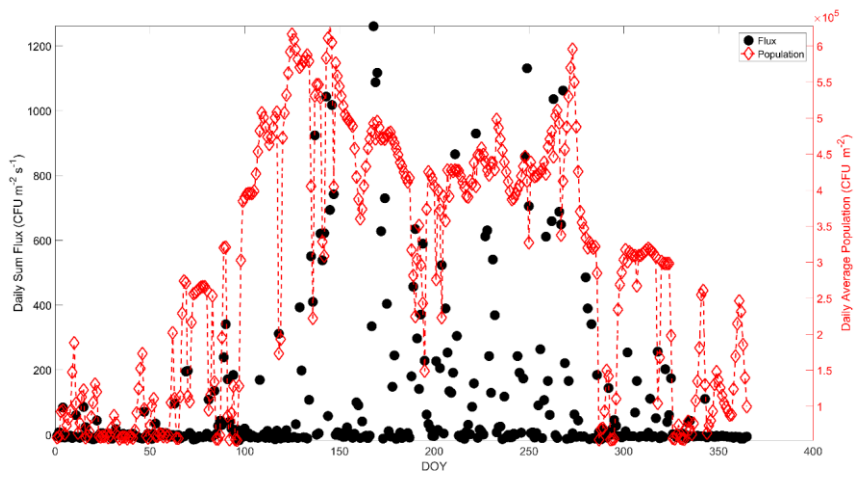


Figure 78: High u^* /low flux event (a) and low u^* /high flux event (b) as simulated for the year 2015. The x-axis indicates the fractional day of the year (DOY, northern hemisphere) for these two events, the left y-axis shows the u^* values, the first right y-axis shows the microbial net flux, and the second right y-axis shows the phyllospheric population size.

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5 **Figure 89:** Time series of the daily sum of modeled microbial fluxes and daily averages of the surface microbial population for 2015. The labels on the x-axis define the DOY starting from 1 January 2015 (DOY 1, northern hemisphere winter) to 31 December 2015 (DOY 365, northern hemisphere winter).

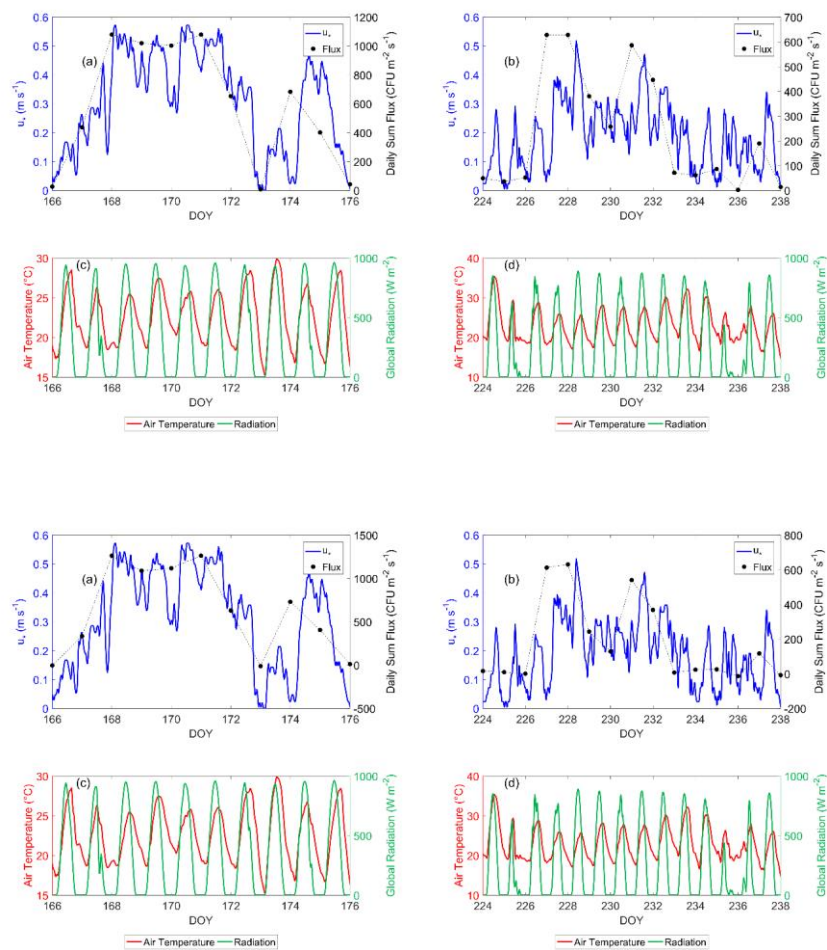


Figure 910: High-wind events in Montfavet, France. Plots a) and b) show friction velocity (grey line, left y-axis) and the daily sum of flux (black dots and dashed line, right y-axis) for two high-wind events (DOY 166-176 and 224-238 of 2015). Plots c) and d) show solar radiation (black line, right y-axis) and air temperature (grey line, left y-axis) for the same two events.

Supplementary Material

1 Flux Computations

The flux-gradient methodology assumes that, in the atmospheric surface layer, the flux of a certain scalar is a function of: the gradient of the said scalar measured at two heights, the delta between the measurement heights, and an appropriate eddy-diffusivity coefficient, in a manner analogous to the parametrization of molecular diffusion (see, for example, (Businger, 1986) and (Baldocchi et al., 1988))

The flux of a certain scalar (F_c) can, therefore, be represented as (Eq. (S1)):

$$F_c = -K_c \left(\frac{dc}{dz} \right), \quad (\text{S1})$$

In Eq. (S1) K_c is the eddy-diffusivity coefficient (in $\text{m}^2 \text{s}^{-1}$), dc represents the gradient of concentration and dz the difference between the two sampling heights. When F_c is positive, an outgoing flux is moving from the surface to the atmosphere (and the surface is, therefore, acting as a source of the scalar c), while the opposite is true if the flux is negative (and, in this case, the surface acts as a sink).

By appropriately scaling K_c on the sampling heights and a scale length, flux can be, instead, expressed as the product of a transport velocity and a difference in concentration, following the aerodynamic method ((Monin and Obukhov, 1954); (Simpson et al., 1998)) and the formulation of (Beine et al., 2003), Eq. (S2)):

$$V_c = k \frac{u_*}{\ln\left(\frac{z_2}{z_1}\right) - \Psi_H\left(\frac{z_2}{L}\right) + \Psi_H\left(\frac{z_1}{L}\right)}, \quad (\text{S2})$$

In Eq. (S2) k is the Von Kármán constant (assumed equal to 0.4), u_* represents friction velocity, z_1 the lowermost sampling height, z_2 the uppermost sampling height, L is the Obukhov length and Ψ_H is the universal similarity function represented as Eq. (S3):

$$\Psi_H = \begin{cases} 2 \ln \left[\frac{1 - \sqrt{1 - 16 \frac{z}{L}}}{2} \right] & \text{if } \frac{z}{L} < 0 \\ -17 \left[1 - \exp\left(-0.29 \frac{z}{L}\right) \right] & \text{if } \frac{z}{L} > 0 \end{cases}, \quad (\text{S3})$$

In Eq. (S3) z/L is the stability parameter.

The actual height z , considered in the aforementioned computations should actually be corrected by subtracting the zero plane displacement height, roughly two-thirds of the average canopy height.

In the present work, due to the considerably short and simple canopy, z is quite insensitive to such correction.

2 Eddy- Covariance Quality Check and Relationship with Flux Gradient results

The eddy-covariance (EC) technique was used to evaluate the applicability of flux-gradient (FG) technique for the Montfavet situation during the September 2015 campaign.

- 5 EC data were elaborated with EddyPro 6 and quality-checked following Mauder and Foken (2006). The flagging methodology followed the CarboEurope IP project standards with a flag of 0 indicating best quality fluxes, a flag of 1 fluxes suitable for general analysis and a flag of 2 fluxes to be discarded. In the September campaign where EC and FG were compared 84.85% of fluxes used for comparison obtained a flag of 0, 10.6% a flag of 1 and only 4.54% a flag of 2. The linear regression between EC and FG did not significantly change when removing flag 2 fluxes. The correlation coefficient changed from 0.72 to 0.70 and slope and offset were substantially unchanged (a difference of 0.005 for the slope and of 0.019 for the offset).

10 A two dimensional footprint model from Kljun et al. (2015) was used to investigate the contribution of the grassland to the measured flux. One aggregated footprint per day was generated, each one spanning a domain of a 25*z meter radius around the tower (76 m) with a resolution of 2 m on both the x and y direction.

- 15 Footprint analysis showed that grassland is the main contributor to the footprint with a cumulative 50-60% contribution. Fluxes are also influenced by terrain with various roughness elements that were taken into account by choosing a conservative z_0 of 0.15 m.

- 20 Footprint analysis also allows investigating potential flow distortions due to the presence of the scaffolding by comparing wind direction and quality flags. During the days between the 26th and 30th of September 2015, the main wind direction was blowing through the scaffolding before reaching the sensors. Between the 26th and the 28th of September, no matter the mainly northerly wind direction, all data had a quality flag of 0, which, from the point of view of the Integral Turbulence Test, means a deviation of less than 30% compared to what is predicted based on similarity theory. Larger errors, with discrepancies > 100%, happened only on the 1st of October, where there was a more “frontal” wind direction, where the disturbance from the scaffolding should be, in fact, less. Flag 2 fluxes were already excluded from analysis since, for microbial gradient method, they do correspond to moments where the two Burkard samplers are below the MRG and thus not providing reliable fluxes.

- 25 It is important to note that EC and FG footprints are, for stable stratification, the same when the EC measurements are made at the arithmetic mean of the highest and lowest gradient measurement heights. Conversely, for unstable stratification, the footprints match when the EC measurements are made at the geometric height of the aforementioned heights (Horst, 1999). Given the height differences between the EC and FG systems, the FG footprints should be smaller (Horst, 1999), receiving more contribution from the grassland itself and being less influenced by external influences and roughness elements.

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