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

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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 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 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 to estimate these emissions on the basis of a few easily recovered meteorological parameters (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 the release 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 explore the impact of microbial aerosols on the atmosphere and climate.

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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.,

5   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

10   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 x 10^4 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

15   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 that induced by non-biological ice

20   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 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. 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

25   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

30   emission fluxes from different ecosystems, while Heald and Spracklen (2009) used mannitol as a proxy to evaluate fungal contributions to PBAs. Burrows et 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.

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 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.

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 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*). Profiles of wind speed, air temperature and viable aerosols were made at two heights ($\approx$67 and $\approx$227 cm), while a sonic anemometer (USA-1, Metek, Elmshorn, DE; located at $\approx$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 CO_2 and H_2O gas exchange concurrently by the eddy-covariance and the flux-gradient method (Baldocchi et al., 1988). 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 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 K_2HPO_4, 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.

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

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.

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

In Eq. (1) A indicates the top sampler, B the bottom sampler and σ the standard deviation.

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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;

10 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

15 and a conservative roughness length ($z0 = 0.15$) was chosen (Businger, 1986). This length was adequate to obtain a $z/z0 > 1$ (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).

20 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 Bukard 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 similar herbaceous species such as cocksfoot (*Dactylis glomerata*), ryegrass, tall fescue (*Festuca arundinacea*) and alfalfa (*Medicago sativa*).

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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 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

30 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.

5   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.

10   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 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

15   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.

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

In Eq. (2) F_e is the removal (i.e.: gross upward flux in CFU m^{-2} s^{-1}); $m1$, $m2$ and $m3$ are respectively 23.3, 255 and 22.3 and

20   were derived though a curve fitting to the Lighthart and Shaffer (1994) flux data with FOOTPRINT92 u_* data (Lighthart and Shaffer, 1994); 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 wind speed, instead of friction velocity, is provided as an input, the model calculates u_* using Eq. (3):

$$u_* = k \, u \ln\left(\frac{z}{z0}\right), \tag{3}$$

25   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 $z0$ 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).

30   $$F_d = \begin{cases} 0 \; if \, \frac{V_g}{u_*} \le 0.1 \\ V_g C_a \xi \; if \, \frac{V_g}{u_*} > 0.1 \end{cases} , \tag{4}$$

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In Eq. (4) F_d is the gross deposition flux (in CFU m^{-2} s^{-1}) and C_a is the airborne concentration of microorganisms (in CFU m^{-3}). V_g is calculated following (Kulkarni et al., 2011) (Eq. 5):

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

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 x10^{-6} m, Raisi et al. (2013) and Schlesinger et al. (2006)); Cc is the Cunningham slip correction factor; and η is the air viscosity (1.83 x10^{-5} Pa s, Kulkarni et al. (2011)).

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. (6):

10    $$C_a = p_1 LAI + p2 ,$$
(6)

In Eq. (6) $p1$=26.99 and $p2$=115.9. 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.

15   The actual net PBAPs flux at a given time (F_n) is computed following Eq. (7):

$$F_n = F_e - F_d,$$
(7)

The phyllosphere microbial population (N, see Eq. (2)) is modeled following Eq. (8):

$$N = rN - F_n,$$
(8)

The growth rate r of Eq. (8) is modeled as a temperature-driven process in Eq. (9) (Yan and Hunt, 1999; Yin et al., 1995;

20   Magarey et al., 2005):

$$r = \begin{cases} 0 & if\ T < T_{MIN} \\ \left(\frac{T_{MAX}-T}{T_{MAX}-T_{OPT}}\right)\left(\frac{T-T_{MIN}}{T_{OPT}-T_{MIN}}\right) c & if\ T_{MIN} \leq T \leq T_{MAX} , \\ 0 & if\ T > T_{MAX} \end{cases}$$
(9)

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.

The model includes also two thresholds: a minimum (k_{min}, which is the point at which all the processes find an equilibrium,

25   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 population (N_0), representing the "boundary condition" for the

30   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

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. (10):

$$\varepsilon = |(1 - |s|)| + |o| + |(1 - |r^2|)|, \tag{10}$$

In Eq. (10), 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}).

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

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 ranging between 0.2 and 28.5 CFU m^{-2} s^{-1}. 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= 0.72) and with minimal bias (y = 1.05x – 0.1; RMSE = 0.78) (Fig. 4), thus supporting the reliability of the flux gradient method.

The measurements made between 2008 and 2010, 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. 5a). Wind speed, instead, showed a high consistency with the 2015 campaign, ranging between 0.4 and 5.8 m s^{-1} (mean 2.4 ± 1.4 m s^{-1}, left y-axis Fig. 5b) 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. 5b). Microbial fluxes 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. 5a). 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), 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). Even if some negative fluxes were registered in 2008-2010, these were a minority compared to the positive ones. Indeed, negative fluxes represented only 16.8 % of all the fluxes measured during this period, confirming that the sampling site tended to be a net microbial emitter, rather than a sink.

3.2 Model Calibration

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

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 temperature values regulating the growth curve of the microorganisms are those with the highest impact on model performance, while the minimum population size (k_{min}) seems to 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. 6 for both the calibration set (2008-2010, Fig. 6a) and the validation campaigns (2015, Fig. 6b). The model is consistent between the two campaigns, explaining roughly 60 % of the variance (r^2 for 2008-2010 is 0.59 while r^2 for 2015 is 0.57) and it does it with a minimal offset (0.88 in 2008-2010 and 0.1 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) and slightly overestimate them

during the 2015 field campaigns (slope of the regression 1.05). The model has an RMSE of 5.22 CFU m^{-2} s^{-1} in 2008-2010, while it is 2.64 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. 7a and 7b. When the population falls below the minimum population (k_{min}), even very high turbulence (mean u_* for Fig. 7a is 0.49 m s^{-1}) won't elicit significant upward net fluxes. Conversely, when the microbial population is large (in Fig. 7b above 3 x 10^5 CFU m^{-2}), even low turbulence (mean u_* for Fig. 7b 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.

Simulated daily sums of PBA fluxes for the entire validation year are shown in Fig. 8. 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. 8). 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. 9a, 9b) and clear skies (Fig. 9c, 9d) 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. 9c).

4. Discussion

The results and tools we present here offer a new approach for studying bioaerosols. Previous attempts to understand the impact of PBAs on precipitation tended to simplify the surface-atmosphere transport 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 end-result of all the emission-deposition process. For this reason, we conceived the PLAnET model to estimate 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

5   fluxes Lighthart and Kirilenko (1998)). While both gross upward and downward flux in the PLAnET model could be resolved separately, this does not happen when employing a gradient method, and the presence of a gravitational effect could 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). The value of this ratio did not exceed the critical thresholds neither in the 2008-2010 campaigns, nor in 2015, giving us confidence in the applicability

10   of the gradient method for the observations at the Montfavet site, since they happened during moments where turbulence was sufficient for generating meaningful gradients.

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

15   would 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

20   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). 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

25   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. 6 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 0.4-1.03 and 0.13- 1.97 respectively). This suggests that the main weakness of the model would only be the number

30   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 a scaling to be compared with the work, for example, of Burrows et al. (2009a), Sesartic et al. (2012) and Sesartic et al. (2013). 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

5   biological versus non-biological particles in a sample independently from their viability. 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

10   appropriate for the PBL response time (≤1 h). This is why in the present work Burkardt samplers where chosen: both for their high volumetric flowrate (500 l min^{-1}) and for their virtual impactor nature that is favorable for preserving particle viability. It is important to consider, though, that even if live and dead microorganisms would contribute to cloud-related processes due to their chemical and physical composition, the latter would not matter from an evolutionary perspective. Live cells have a chance of further transmit their biological and chemical footprints to a wider microbial population. The latter

15   would represents 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), 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 of a larger number of different ecosystems, such as

20   forests and agricultural crops.

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 0.26 CFU m^{-2} s^{-1}) versus higher and more

25   variable average fluxes in summer ranging from 3.8 to 8.8 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 2.71 x10^5 CFU m^{-2} versus a seasonal average of 3.81 x10^5 CFU m^{-2} and an average temperature of 26°C versus 23°C. The lowest average summer population size was simulated, instead, in 2006 (2.57 x10^5

30   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,

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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

5   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) is compared against a non-rainy month (September 2007 with an

10   average rainfall of 0.027 mm), average net fluxes are very similar and actually greater in the less rainy month (5.49 CFU m^{-2} s^{-1} in September 2007 versus 4.55 CFU m^{-2} s^{-1} in September 2010).

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 has been estimated through a sound

15   micrometeorological method (flux-gradient methodology). 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. The latter statement is true when colonies behave as a passive tracer and the lack of significant aerodynamic effects was therefore tested for the

20   experimental conditions using literature relationships between friction velocity and size of transported particles. The grassland showed a majority of 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 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

25   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 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

30   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 is nested and generate time-varying

emission rates at surface 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,

5   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 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*	22.3-23.3	Derived by the authors from Lighthart and Shaffer (1994)	None
m_2*	250-260	Derived by the authors from Lighthart and Shaffer (1994)	None
m_3*	21.3-23.3	Derived by the authors from Lighthart and Shaffer (1994)	None
d	1800	Equation (6)	Seconds
p_1	26.99	Derived by the authors	None
p_2	115.9	Derived by the authors	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.

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	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	5×10^4	4.82×10^6	22.97	250.06	21.3
Sensitivity	1.16	0.40	0.24	0.03	0.31	0.30	0.02	0.19

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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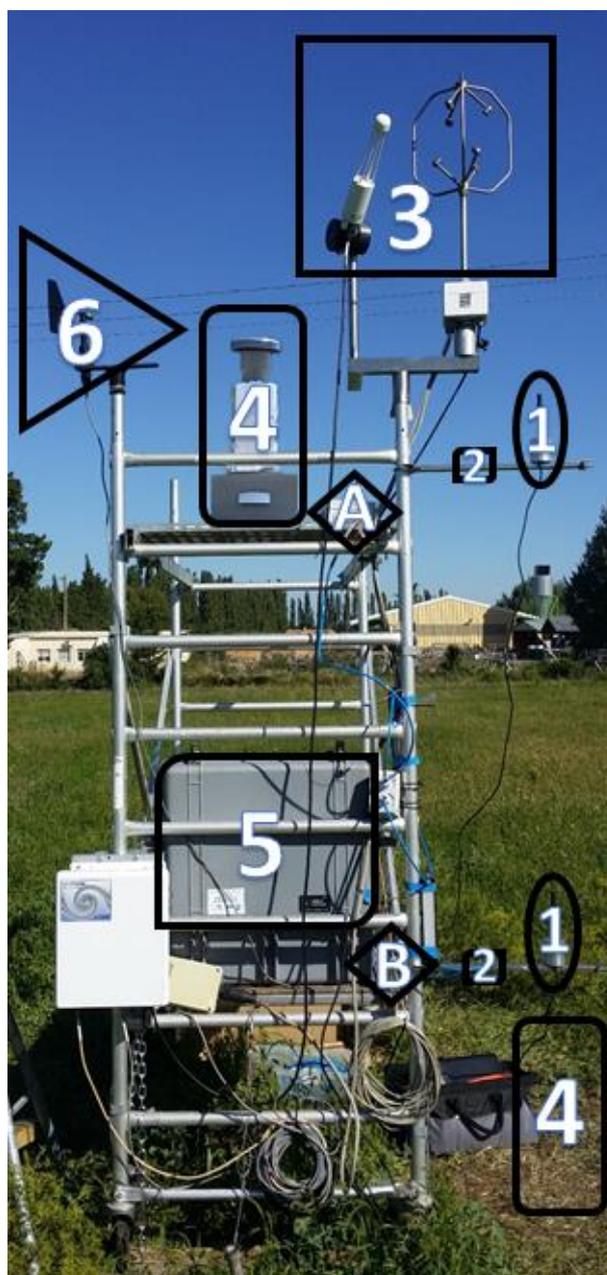
Figure 1: Schematic representation of the sampling station: each piece of equipment is represented by a number (in bold face) and the position of the equipment, expressed as cm a.g.l is indicated in parenthesis. Cup anemometers (**1**; 80 and 210) , thermocouples (**2**; 80 and 210), sonic anemometer 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).
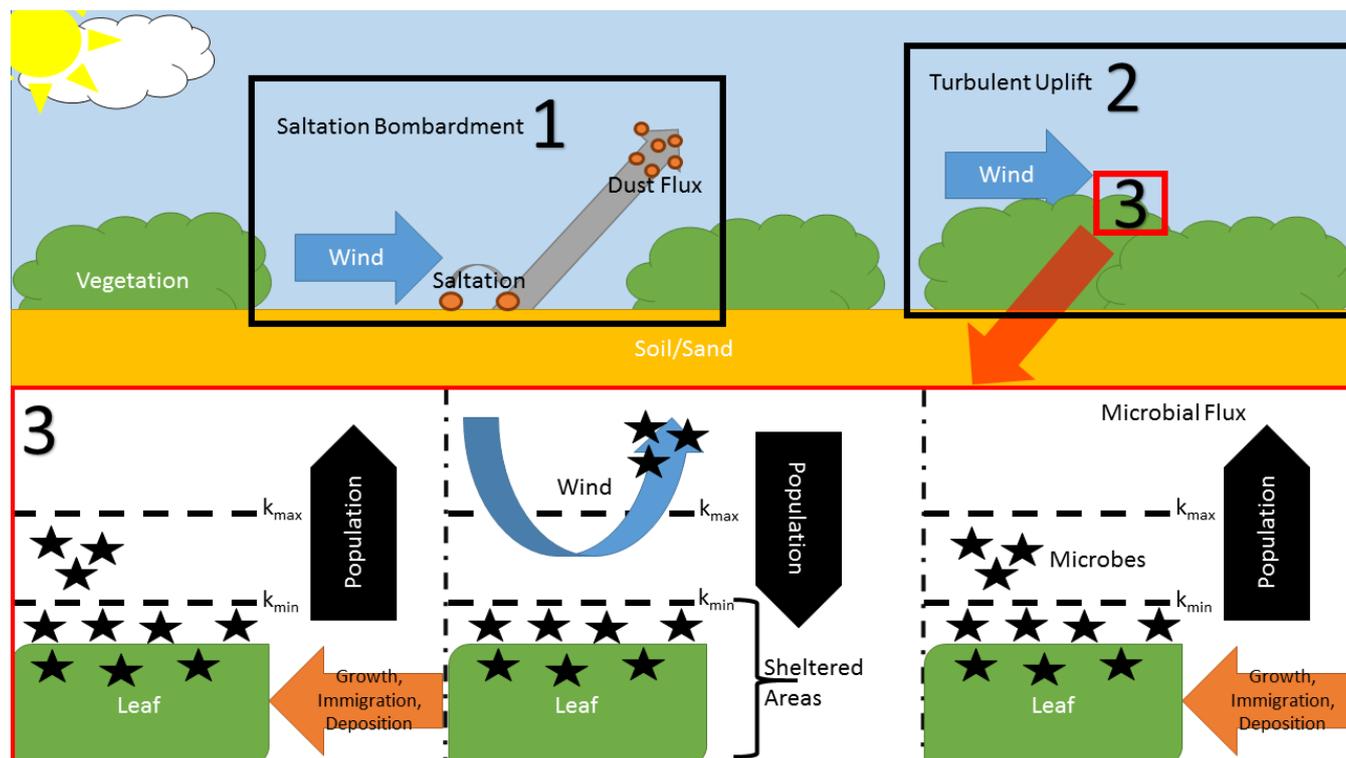
Figure 2: Schematics of the differences between Aeolian dust flux and microbial flux on which the PLAnET model is founded. Box 1(top left) shows the typical dust saltation mechanism. The action of wind (blue arrow) on soil makes dust particles (orange dots) "jump" for short distances, ejecting smaller 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.
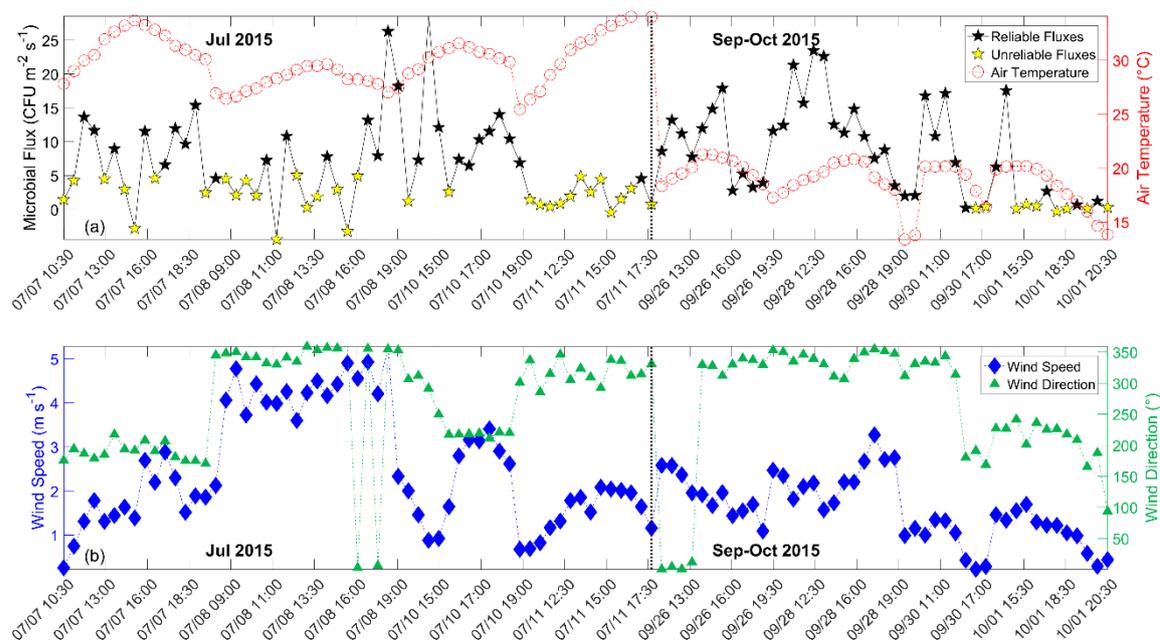
Figure 3: Dynamics of observed microbial fluxes, air temperature, wind speed and wind direction in the 2015 field campaigns. Fig. 3a shows the time series of air temperature and microbial fluxes for July and September-October 2015. Unreliable fluxes are those below the MRG. Fig. 3b shows the time series 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).
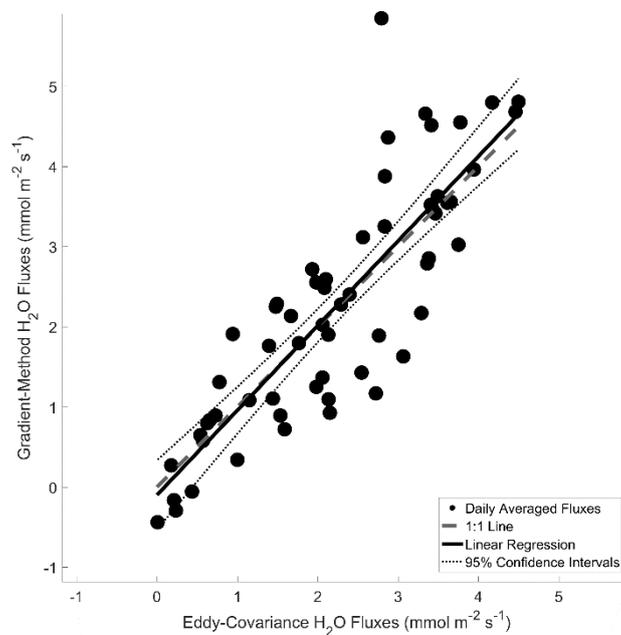
Figure 4: Water vapor fluxes measured via the eddy covariance method (x-axis) *versus* those derived from the flux-gradient method (y-axis). The plotted linear regression has a slope of 1.05, an offset of 0.1 and explains 72% of the data variability.
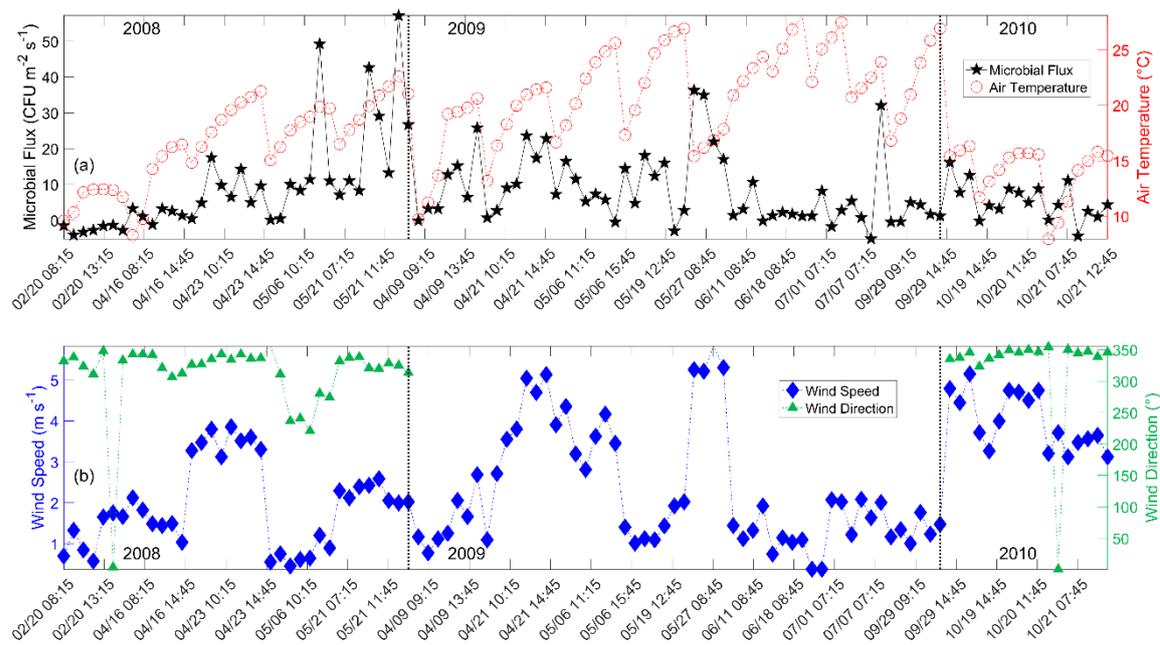
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Figure 5: Temporal dynamics of observed microbial fluxes, air temperature, wind speed and wind direction in the 2008, 2009 and 2010 field campaigns. Fig. 5a shows time series of air temperature and microbial fluxes for the campaigns held between 2008 and 2010. Fig. 5b 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.

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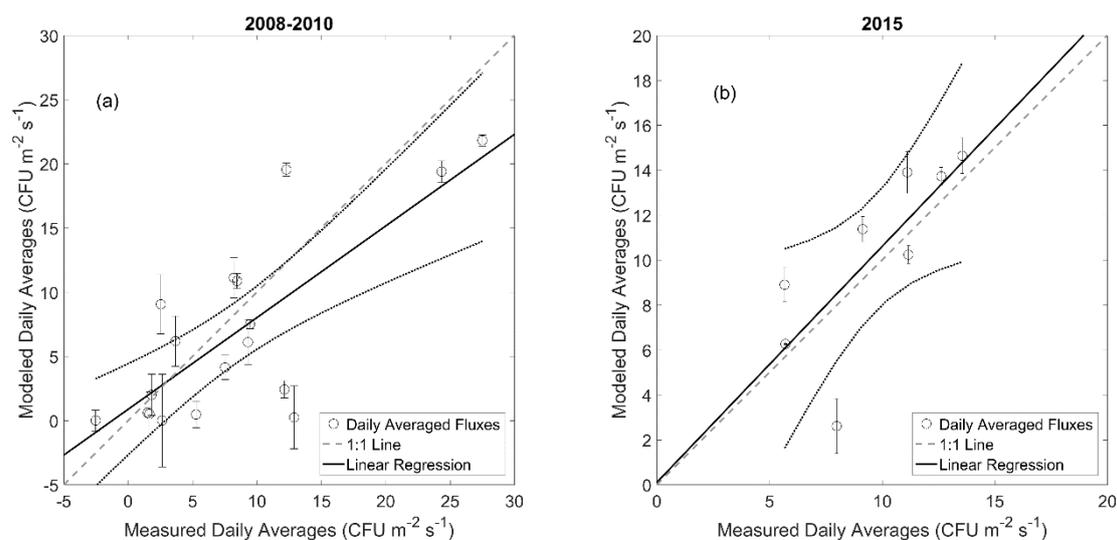Chemistry
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Figure 6: Relationship between measured and modeled daily averages of microbial flux. Fig. 6a shows the regression between the daily averages from the 2008-2010 campaigns and the optimized model (y=0.71x+0.88; r^2=0.59) and Fig. 6b shows the one from the 2015 campaigns and the model (y=1.05x+0.11; r^2= 0.57). 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 ($\overline{m_i, o_i}$).

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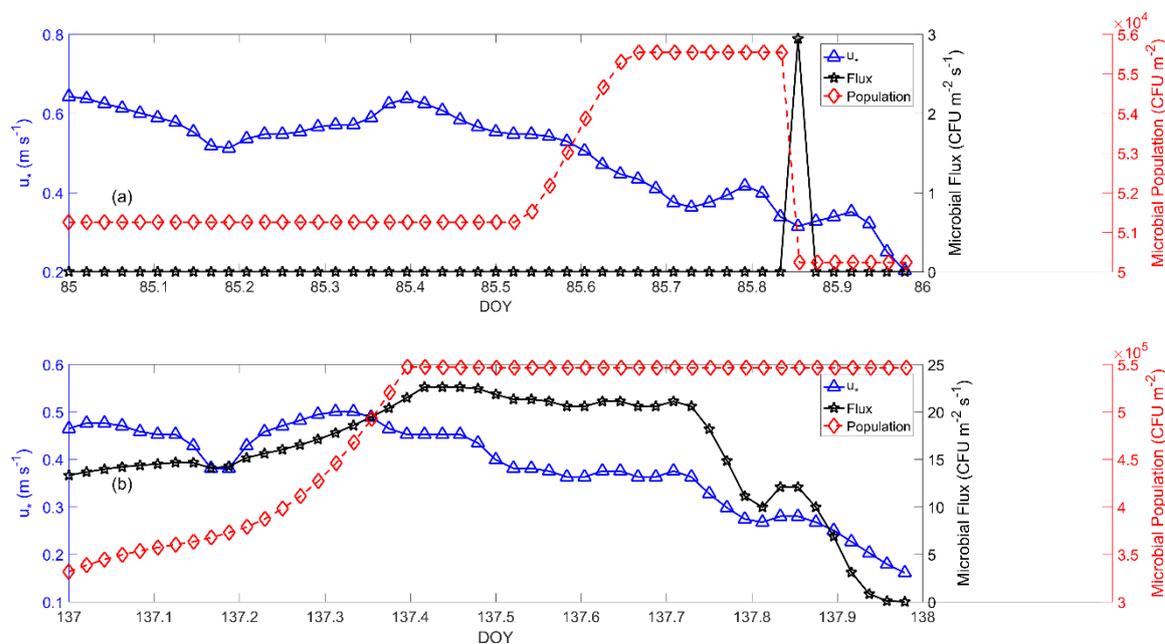Chemistry
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Figure 7: High u∗/low flux event (a) and low u∗/high flux event (b) as simulated for the year 2015. The x-axis indicates the fractional day of the year (DOY, northern hemisphere) for these two events, the left y-axis shows the u∗ values, the first right y-axis shows the microbial net flux, and the second right y-axis shows the phyllospheric population size.
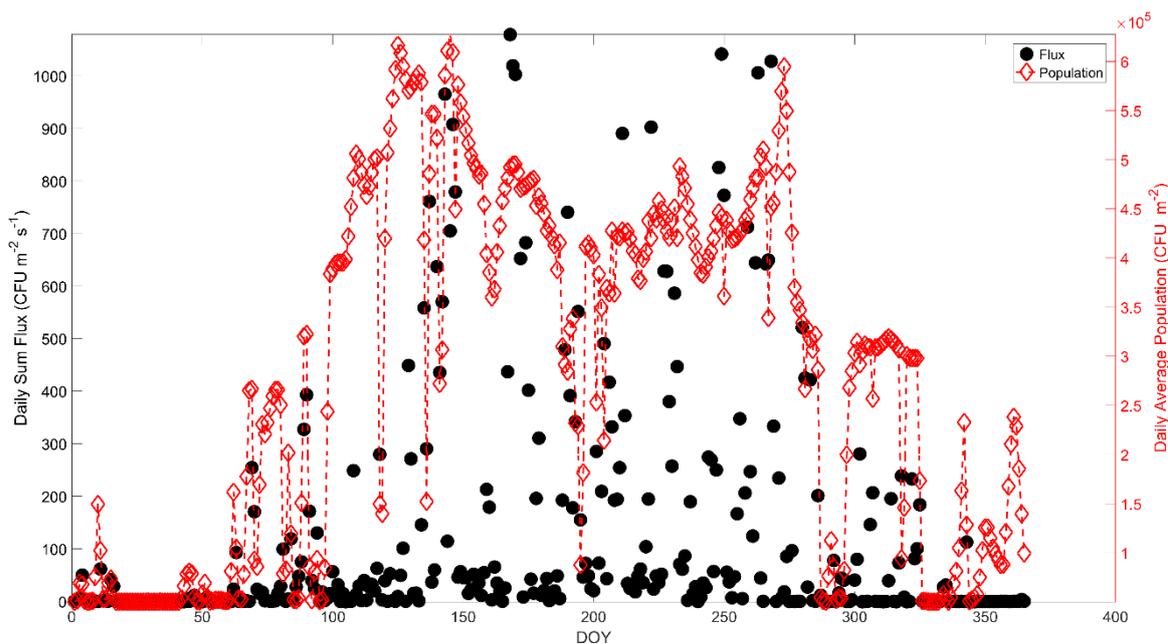
Figure 8: Time series of the daily sum of modeled microbial fluxes and daily averages of the surface microbial population for 2015. The labels on the x-axis define the DOY starting from 1 January 2015 (DOY 1, northern hemisphere winter) to 31 December 2015 (DOY 365, northern hemisphere winter).
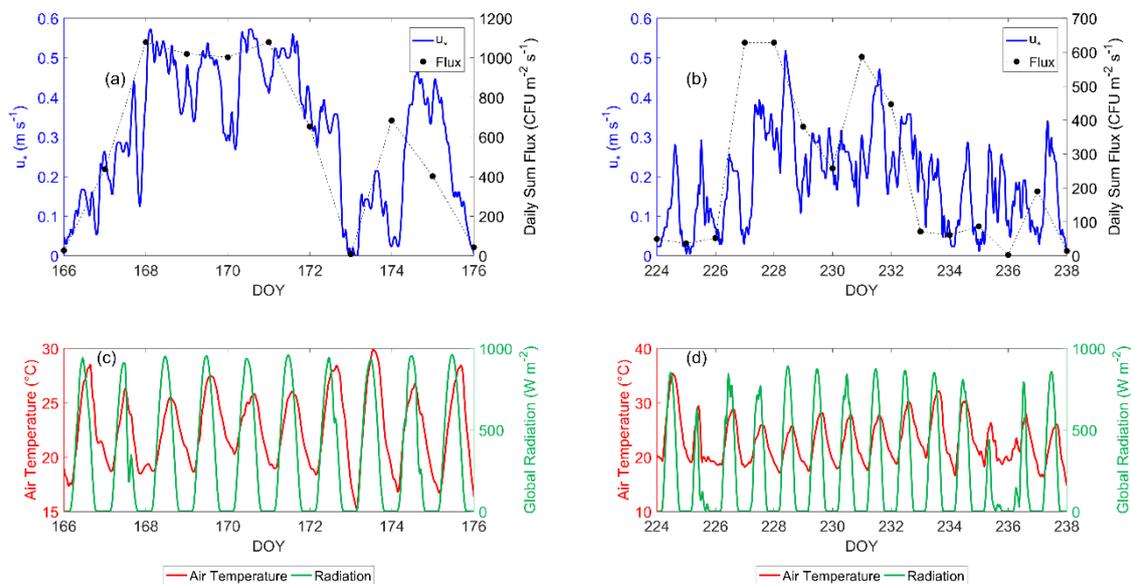
Figure 9: High-wind events in 2015 in Montfavet, France. Plots a) and b) show friction velocity (grey line, left y-axis) and the daily sum of flux (black dots and dashed line, right y-axis) for two high-wind events (DOY 166-176 and 224-238 of 2015). Plots c) and d) show solar radiation (black line, righy y-axis) and air temperature (grey line, left y-axis) for the same two events.

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