Potential impact of microbial activity on the oxidant capacity and organic carbon budget in clouds

Mickael Vaïtilingom[a,b,c,d], Laurent Deguillaume[c,d], Virginie Vinatier[a,b], Martine Sancelme[a,b], Pierre Amato[a,b], Nadine Chaumerliac[c,d], and Anne-Marie Delort[a,b,1]

[a]Institut de Chimie de Clermont-Ferrand and [c]Observatoire de Physique du Globe de Clermont-Ferrand, Laboratoire de Météorologie Physique, Clermont Université, Université Blaise Pascal, BP 10448, F-63000 Clermont-Ferrand, France; [b]Centre National de la Recherche Scientifique, Unité Mixte de Recherche 6296, Institut de Chimie de Clermont-Ferrand, BP 80026, F-63171 Aubière, France; and [d]Centre National de la Recherche Scientifique, Unité Mixte de Recherche 6016, Laboratoire de Météorologie Physique/Observatoire de Physique du Globe de Clermont-Ferrand, BP 80026, F-63177 Aubière, France

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Within cloud water, microorganisms are metabolically active and, thus, are expected to contribute to the atmospheric chemistry. This article investigates the interactions between microorganisms and the reactive oxygenated species that are present in cloud water because these chemical compounds drive the oxidant capacity of the cloud system. Real cloud water samples with contrasting features (marine, continental, and urban) were taken from the puy de Dôme mountain (France). The samples exhibited a high microbial biodiversity and complex chemical composition. The media were incubated in the dark and subjected to UV radiation in specifically designed photo-bioreactors. The concentrations of H_2O_2, organic compounds, and the ATP/ADP ratio were monitored during the incubation period. The microorganisms remained metabolically active in the presence of $^{\bullet}OH$ radicals that were photoproduced from H_2O_2. This oxidant and major carbon compounds (formaldehyde and carboxylic acids) were biodegraded by the endogenous microflora. This work suggests that microorganisms could play a double role in atmospheric chemistry; first, they could directly metabolize organic carbon species, and second, they could reduce the available source of radicals through their oxidative metabolism. Consequently, molecules such as H_2O_2 would no longer be available for photochemical or other chemical reactions, which would decrease the cloud oxidant capacity.

biodegradation | cloud chemistry

The cloud system is an ideal medium for the development of complex multiphase chemistry, in which chemical species from the gas, solid, and aqueous phases are transformed. This process perturbs the homogeneous gas phase chemistry through the dissolution of various chemical compounds that undergo efficient photochemical processing. During a cloud's lifetime, cloud chemistry can lead to the formation of new, low volatile compounds, such as organic and inorganic acids, that modify the physical and chemical properties of aerosols after cloud evaporation and can also contribute to the formation of secondary aerosols (1, 2). The formation of clouds is, consequently, modified, and this process remains one of the major uncertainties in climate models that assess the earth's radiative balance (3).

Within this framework, the presence of free radicals and oxidants in the cloud system leads to aqueous phase oxidations, transforming both inorganic and organic compounds. Cloud chemistry models predict that the $^{\bullet}OH$ radicals represent the most important oxidant in the cloud aqueous phase (4). This oxidant can either be transferred from the gas phase or produced in situ in the aqueous phase through photochemical processes or related reactions with hydrogen peroxide and transition metal ions, such as iron (5). Multiple other oxidants that are produced in clouds can also oxidize chemicals, and these oxidation processes must be better understood because they impact atmospheric chemical cycles and radiation. Indeed, the resulting aerosols increase or decrease the scattering albedo, thus modifying the radiative forcing by clouds.

Many volatile organic compounds from secondary formations are associated with moderately high Henry's law constants and are, consequently, dissolved into the tropospheric aqueous phase (6). Additionally, organic compounds constitute a significant mass fraction of tropospheric aerosol particles, which can also be transferred into cloud water. Hence, the dissolved organic matter is able to interact directly or indirectly with the aqueous chemistry of radicals, radical anions, nonradical oxidants, and transition metal ions. A large proportion of the dissolved organic matter is still not characterized, but carboxylic acids could represent a significant proportion of this soluble matter. Among these acids, the formic and acetic acids are the most abundant (mainly produced in the gaseous phase), oxalic acid is commonly the third dominant species and the main di-carboxylic acid, followed by succinic, malonic, and maleic acids (predominantly dissolved from organic particles) (7–10).

Cloud water also hosts microbial populations that are primary biological aerosols and the dominant living aerosols that are present in the atmosphere (11–14). These aerosols can be integrated into clouds because they can serve as cloud condensation nuclei for droplet formation (13–15). This environment is stressful for airborne microorganisms (low temperature, desiccation, oxidation, UV radiation, acidic pH in the aqueous phase, and so forth) (13, 16). Low temperatures appear to represent one of the major obstacles for cellular activity because they are directly linked to decreased molecular motion and reaction rates. However, bacteria can sustain growth in cloud water at temperatures at or below 0 °C (17), and they are capable of maintaining metabolic activity at subzero temperatures down to −20 °C (18–20). Cultivable microorganisms (fungal spores, yeasts, and bacteria) have been found in fog and cloud water (21–23). Bauer et al. demonstrated that the majority of bacteria that are present in cloud water are viable (up to 95% in two samples) (21). The ATP concentrations in cloud water that were measured by Amato et al. (24) suggest that a significant fraction of the microorganisms that are present in these environments are metabolically active.

The discovery of microbial activity in this environment has indicated that there are biologically mediated processes in the chemistry of clouds. In the recent past, researchers investigated the microbial activity in cloud water containing organic compounds, such as carboxylic acids, formaldehyde, and methanol (25, 26). Inferred estimates indicated that the activity of microorganisms was likely to affect the chemistry of these compounds in warm clouds and could even drive their reactivity during the night (27–29).

All of these studies are pertinent, but they were conducted under conditions that were different from those in real clouds. In particular, the presence of reactive oxygenated species, such as hydrogen peroxide (H_2O_2) and free radicals, was ignored.

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[1]To whom correspondence should be addressed. E-mail: A-Marie.DELORT@univ-bpclermont.fr.

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Although these compounds are toxic to cell life, the survival of microorganisms in clouds strongly suggests that cloud-borne microorganisms can resist the high concentrations that are found in the atmosphere. This result is likely to be because of the efficient, antioxidative stress metabolism that involves specialized enzymes (such as catalases, peroxidases, and superoxide dismutase) and nonenzymatic compounds (30, 31).

We investigated the metabolic activity of microorganisms in microcosms that were more similar to the real cloud environment. We used cloud water samples that were collected at the puy de Dôme mountain, which is a reference site in France for cloud observations and is part of the European ACTRIS (Aerosols, Clouds, and Trace gases Research InfraStructure Network) project. The samples contained complex mixtures of organic and inorganic compounds, oxidants, such as iron complexes and H_2O_2, and the endogenous microflora. The cloud water samples were either kept intact or were sterilized by filtration and were then incubated in the dark or subjected to UV-light radiation in specially designed photo-bioreactors (Fig. S1). This procedure resulted in the separation of biologically driven processes and other chemical phenomena (including photochemistry) that occurred in the cloud water under controlled conditions. The biological and chemical characterizations were conducted throughout the incubation. Under these conditions, the microorganisms were exposed to H_2O_2 and potential $^{\bullet}OH$ radical photoproduction. This study had four specific objectives: (*i*) to study the possible biodegradation processes of H_2O_2; (*ii*) to determine the energetic state (ADP/ATP ratios) of cells under these stressful conditions; (*iii*) to investigate the impact of the presence of reactive oxygenated species on the biodegradation rates of organic acids (acetate, formate, oxalate, malonate, and succinate) and formaldehyde; and (*iv*) to compare abiotic and biotic processes.

The major result of this work helps to answer two questions: (*i*) Do microorganisms interact with reactive oxygenated species? and (*ii*) Consequently, does microbial activity control the oxidant capacity and the organic carbon budget in natural clouds?

Results and Discussion

Three cloud events were sampled in June 2010 at puy de Dôme mountain (1,465 m above sea level). The backward trajectories from the National Oceanic and Atmospheric Administration Hysplit model were plotted for the various sampled air-masses and are displayed in Fig. S2. Three cloud types were selected for their contrasting features: cloud 1 had a northwestern marine origin, cloud 2 was from the continental southwest, and cloud 3 was from the continental northeastern flux but was influenced by anthropogenic emissions. The average temperatures during sampling were 10 °C for clouds 1 and 3 and 13.5 °C for cloud 2 (see Table 1 for additional physico-chemical parameters).

Chemical and Biological Content of Cloud Water Samples. The chemical and biological data from the three cloud water samples are summarized in Table 1. The chemical properties of these three samples are consistent with their respective origins. For example, cloud 3, collected from an "urban" air-mass, was more acidic and oxidant (pH = 3.9 and $[H_2O_2]$ = 57.7 μM) than cloud 1, which had a "marine" origin (pH = 6.1 and $[H_2O_2]$ = 3.6 μM). Cloud 2, from the "continental" air-mass, represented an intermediate condition.

The five most abundant carboxylic acids that are usually found in cloud water [i.e., formic, acetic, oxalic, succinic, and malonic acids (7–9)] and the most abundant aldehyde (i.e., formaldehyde) were present in clouds 2 and 3, but succinic and malonic acids were not detected in cloud 1. The contribution of carboxylic acids and formaldehyde to dissolved organic carbon was ~19%, 25%, and 23% in clouds 1, 2, and 3, respectively. The total number of microbial cells was of the same order of magnitude as the typical previous measurements at the puy de Dôme site (23, 32) and was very similar to samples from Mt. Rax in Austria (1,644 m above sea level) (21).

Table 1. Initial bio-physico-chemical characteristics for the three cloud events, sampled at the puy de Dôme station

Characteristic	Cloud 1	Cloud 2	Cloud 3
Air-mass origin	Northwestern	Southwestern	Northeastern
Air-mass type	Marine	Continental	Urban
Date of sampling	6/1/10 8:20 PM	6/8/10 12:05 PM	6/18/10 11:15 AM
Duration of sampling	6:30	11:20	19:45
Temperature	10 °C	13.5 °C	10 °C
pH	6.1	5.2	3.9
Conductivity ($\mu S \cdot cm^{-1}$)	3.5	37.6	78.6
TOC (DOC) ($mg \cdot L^{-1}$)	1.1 (1.1)	6.8 (6.7)	6.9 (6.8)
Compound	Concentration (μM)		
Acetate	4.5	25.4	23.2
Formate	4.9	42.7	33.2
Succinate	—	3.1	3.8
Oxalate	1.0	9.7	9.3
Malonate	—	3.1	3.5
Cl^-	3.0	7.7	11.3
NO_3^-	4.5	70.6	228.7
SO_4^{2-}	1.8	46.1	64.0
Na^+	2.2	10.1	8.8
NH_4^+	8.5	100.3	122.3
K^+	—	1.5	2.2
Mg^{2+}	1.0	2.1	2.7
Ca^{2+}	1.7	3.8	3.8
Fe (total)	0.9	1.1	1.3
Fe (II)	0.3	0.5	0.5
Formaldehyde	1.5	2.7	6.1
H_2O_2	3.6	33.4	57.7
ATP ($pmol \cdot mL^{-1}$)	0.8	2.3	2.1
ADP ($pmol \cdot mL^{-1}$)	1.1	0.7	1.1
ADP/ATP ratio	1.4	0.3	0.5
Total fungal spores and yeasts ($cells/mL^{-1}$)	9×10^3	3×10^3	3×10^3
Total bacteria ($cells/mL^{-1}$)	3×10^4	8×10^4	9×10^4

DOC, dissolved organic carbon; TOC, total organic carbon.

Hydrogen Peroxide Biotransformation in Real Cloud Water Microcosms. The three cloud water samples were incubated at 17 °C under four incubation regimes: unfiltered and in the presence or absence of UV radiation ("Microorganisms + Light" and "Microorganisms," respectively) and filtered and in the presence or absence of UV radiation ("Light" and "Reference," respectively). H_2O_2 concentrations were measured periodically over the incubation period and are plotted in Fig. 1; the corresponding degradation rates are reported in Table 2.

In the absence of UV radiation in filtered cloud water (Reference), a slow degradation of H_2O_2 was observed in clouds 2 and 3. This phenomenon can be explained by the reactivity of H_2O_2 with chemical species, such as transition metal ions (a.k.a. "Fenton reactions") or sulphite (33, 34). The zero-order kinetic constants were the same for the two clouds, most likely because of the very similar iron concentrations. In cloud 1, the degradation of H_2O_2 was even slower, most likely because the species responsible for the radical reactions (S and Fe) were present at lower concentrations than in clouds 2 and 3 (Table 1). Under UV light, the first-order kinetics had similar constants, indicating that H_2O_2 was photolyzed, producing $^{\bullet}OH$ radicals. Interestingly, in the presence of microorganisms without UV light, H_2O_2 was also efficiently degraded in cloud 2 ($v_c = 28.5 \times 10^{-11}$ $M \cdot s^{-1}$) and cloud 3 ($v_c = 30.0 \times 10^{-11}$ $M \cdot s^{-1}$). In cloud 1, the microbial

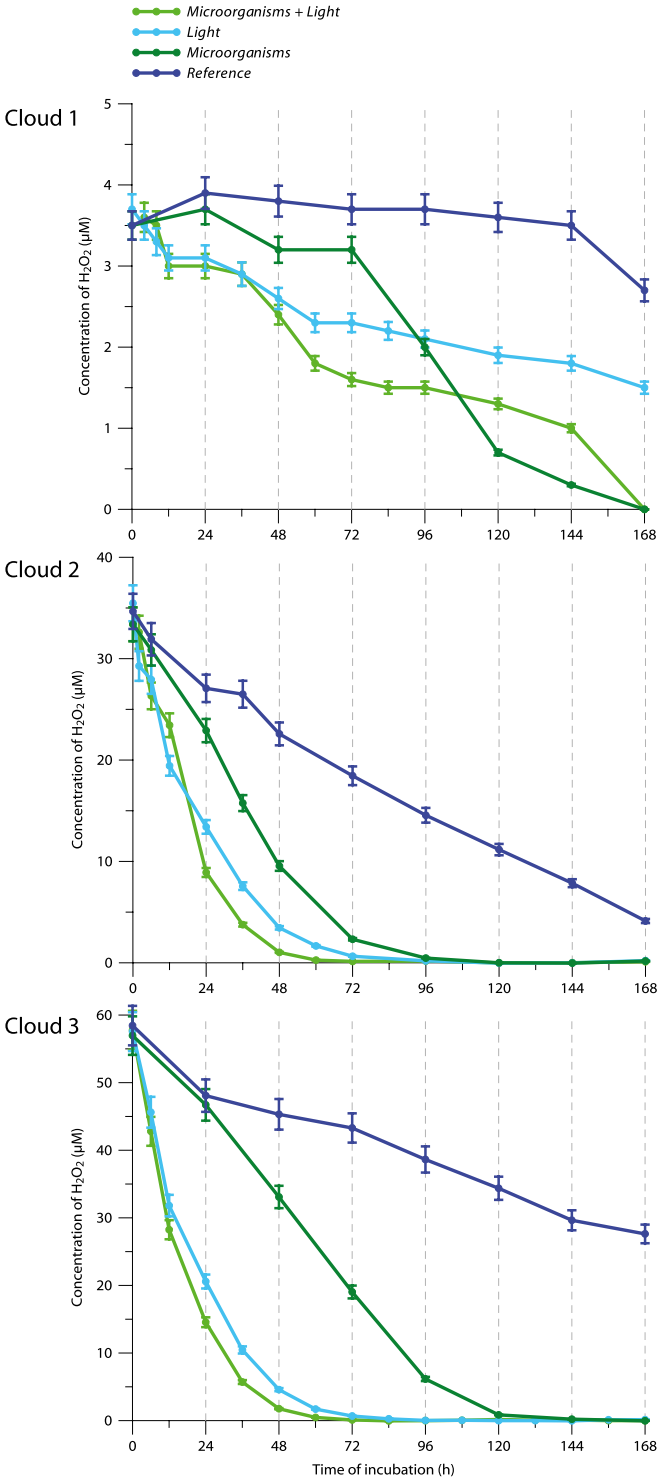

Fig. 1. Temporal evolution of H_2O_2 concentrations (μM) in the presence or absence of UV light or microorganisms during incubation of cloud water (clouds 1, 2, 3). Cloud water samples were incubated at 17 °C under four incubation regimes for 7 d: unfiltered and in the presence or absence of UV radiation (Microorganisms + Light and Microorganisms, respectively), filtered and in the presence or absence of UV radiation (Light and Reference, respectively). Error bars represent the SEs of the enzymatic assay (5%).

degradation of H_2O_2 was very slow until 72 h but then increased ($v_c = 2.9 \times 10^{-11}$ M·s^{-1}). In darkness, the presence of microorganisms enhanced the rate of H_2O_2 degradation by a factor of 2.9 and 4.2 for clouds 2 and 3, respectively. Combining UV light and microorganisms resulted in higher degradation rates for H_2O_2 than either individual condition alone. For clouds 2 and 3, the degradation rates for H_2O_2 appeared to be additive: Light and Microorganisms ~ Microorganisms + Light (Table 2). These results demonstrate that cloud microorganisms can metabolize H_2O_2 into O_2 and H_2O using catalases, which are ubiquitous oxidoreductase enzymes.

Table 2. Initial degradation rates of H_2O_2 in the presence and absence of UV light or microorganisms during the incubation of natural cloud waters

	Cloud 1	Cloud 2	Cloud 3
Incubation regime	Rate of H_2O_2 transformation ($\times 10^{-11}$ M·s^{-1})		
Reference*	0	−9.8	−7.2
Light	−0.6	−48.6	−105.1
Microorganisms	−2.9 (72 h to end)	−28.5	−30.0
Microorganisms + Light	−0.9	−68.4	−126.9

A negative value indicates the disappearance of H_2O_2 from the medium. In the case where a noncontinuous transformation occurred, the time period used for the linear regression is indicated in brackets.
*Reference: sterilized sample (filtration 0.22 μm) incubated in darkness.

Furthermore, it was possible to quantify the relative impact of biotic activity vs. abiotic H_2O_2 transformations (Fig. S3 and Tables S1 and S2). During the day, three types of H_2O_2 degradation mechanisms were active in clouds 2 and 3. Photodegradation was the major process (54% and 76%, respectively), followed by biodegradation (30% and 18%, respectively). Other abiotic reactions accounted for 16% in cloud 2 and 6% in cloud 3. In cloud 1, only light was involved initially. During the night, photodegradation was no longer involved, and microbial activity was the major process, accounting for 66% (cloud 2) and 76% (cloud 3) of H_2O_2 degradation; nonphotochemical abiotic reactions only accounted for 34% (cloud 2) and 24% (cloud 3). These reactions were too slow to be measured in cloud 1 during the first few hours. Clearly, the impact of microbial activity controlling the oxidant capacity in warm clouds may be important, particularly at night, when it may be dominant.

In addition, the results obtained during the combined photobiodegradation processes indicate that photoproduced radicals are not toxic to cloud microflora; this statement is consistent with the oxidative stress metabolism of microorganisms. This type of metabolism involves not only catalases but also peroxidases and superoxide dismutase, as well as other nonenzymatic antioxidants (31).

Microbial Energetic States in Real Cloud Water Microcosms. In the previous section, we noted that microorganisms were exposed to photoproduced radicals, which are known to be potentially toxic to microbial cells. Therefore, we investigated the ability of cloud microorganisms to resist these oxidative stresses by quantifying the ADP/ATP ratios during the same incubations. Essentially, growing bacteria present a ratio of ~0.25, whereas dead cells have a ratio >6 (35). The plots presented in Fig. 2 reflect the evolution of the energetic states of these microorganisms over time.

In all cases, the microbial energetic state improved over time as the ADP/ATP ratios decreased. The microorganisms in clouds 2 and 3 had similar initial energetic states (ADP/ATP ratios of 0.3 and 0.5, respectively) that decreased slightly over the incubation period and reached 0.1 and 0.2, respectively, after 72 h. The initial energetic state of bacteria from cloud 1 was lower (ADP/ATP ratio 1.4) than the other cloud samples. This difference could be because of some specific, unfavorable stress that was encountered by the microorganisms during the air-mass history. The ADP/ATP ratio increased over time and attained an energetic state that was similar to clouds 2 and 3 after 72 h. This recovery of a higher energetic state after 72 h reflects an activation of the microbial metabolism. Consequently, the biodegradation

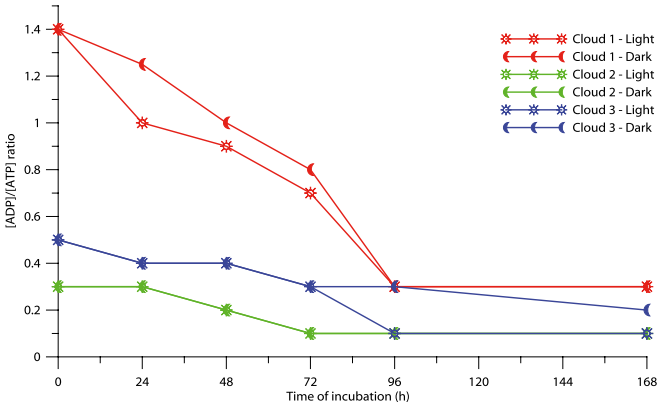

Fig. 2. [ADP]/[ATP] ratios of microbial cells in the presence and absence of UV light during the incubation of unfiltered cloud water samples (clouds 1, 2, 3).

rate of H_2O_2 increased, as displayed in Fig. 1 (cloud 1), when microorganisms were present in the incubation medium.

The ADP/ATP ratios during the microbial incubations under dark and UV light conditions were similar. Clearly, photodegradation in the presence of H_2O_2, notably the production of $^{\bullet}OH$ radicals under light conditions, did not affect the cells' energy metabolism. This result is in agreement with the additive nature of the photochemical and biological processes that are involved in H_2O_2 degradation (Table 2).

Impact of the Presence of Reactive Oxygen Species on the Biotransformation of Carboxylic Acids and Formaldehyde in Real Cloud Water Microcosms. Formate, acetate, succinate, oxalate, malonate, and formaldehyde were also measured in the four incubation regimes that were used for H_2O_2. Focusing on cloud 2, the evolution of the concentrations of carboxylic acids and formaldehyde are plotted in Fig. 3, and the production and degradation rates of carboxylic acids and formaldehyde are presented in Table 3. Because they are very similar to cloud 2, the results for the other clouds are presented in Figs. S4 and S5. Acetate, formate, and succinate were only degraded in the presence of microorganisms, and oxalate was only degraded in the presence of UV light. Malonate and formaldehyde were photoproduced during the experiment and degraded in the presence of microorganisms. The biotransformation was not delayed for acetate, succinate, malonate, and formaldehyde, but in the case of formate we observed a lag time (up to 48 h) before the biodegradation began. The degradation of oxalate by UV light in the absence of microorganisms indicated that photochemical reactions were involved. This conclusion is consistent with the concomitant H_2O_2 photolysis under the same conditions. It is also important to note that the degradation rates in the Reference sample were close to zero, demonstrating that the major processes were photodegradation and biodegradation.

The most interesting results clearly show that the endogenous microflora were not inhibited by the presence of reactive oxygen species (H_2O_2 and photoproduced $^{\bullet}OH$ radicals). In the case of acetate, formate, and succinate, the degradation rates that were measured for Microorganisms (15.5, 17.5, and 4.5×10^{-11} M·s^{-1}, respectively) and Microorganisms + Light (15.6, 16.1, and 3.5×10^{-11} M·s^{-1}, respectively) were very similar. Therefore, the presence of light, and thus of $^{\bullet}OH$ radicals, had no influence on microbial carbon metabolism. In the case of formaldehyde, the photoproduction rate was 0.2×10^{-11} M·s^{-1} (Light), and the biodegradation rate (Microorganisms) was 0.3×10^{-11} M·s^{-1}. When the two processes were combined (Microorganisms + Light), the resulting rate of transformation was null, indicating that the addition of the processes occurred without any inhibition. The case of malonate is rather similar to that of formaldehyde; the rate observed during the combined photo-biodegradation processes after 36 h (4.3×10^{-11} M·s^{-1}) corresponded to the addition of the photoproduction rate (0.3×10^{-11} M·s^{-1}) and the biodegradation rate (4.2×10^{-11} M·s^{-1}). The conclusions related to cloud 2 can be extended to clouds 1 and 3 (see comments in the *SI Text*); they are straightforward in the case of cloud 3, but the case of cloud 1 is more complex to interpret. This difficulty in interpretation could be because of the unusually low concentration of organic and inorganic compounds in cloud 1 (Table 1) and to the lower initial energetic state of the microorganisms (Fig. 2), which explains why biodegradation only began after 72 h. Global comments about the three clouds are provided in the *SI Text* and are related to Figs. S4 and S5 and Table S3, which lists all of the transformation rates.

The principle of the noninhibition of reactive oxygen species to the biodegradation process was observed in all of the study clouds, and the addition of the various photo- and biotransformation rates remains valid (*SI Text*). This result has major consequences when considering the impact of microbial activity on carbon budgets; it suggests that microorganisms could play a role not only in cloud chemistry at night, as previously indicated (27–29), but also during the daytime, when $^{\bullet}OH$ radicals are photoproduced. The implication of microorganisms in carbon flux in the atmosphere at the global scale was estimated (*SI Text* and Table S4). This rough calculation results in a global release of 51–215 million tons of CO_2 per year through microbial respiration.

Conclusions

We studied microbial activity in real cloud samples that represent the three major categories of air-masses on the puy de Dôme mountain (marine, continental, and urban origins). Their physical and chemical compositions thus represented varied experimental scenarios, and the endogenous microflora in each sample were likely to be different. Indeed, the microbial composition of cloud waters greatly depends on the sources of microbial aerosolization (vegetation, oceans, urban areas, and so forth) that are present on the air-mass trajectories and also on

Table 3. Initial transformation rates of carboxylic acids and formaldehyde in the presence and absence of UV light and/or microorganisms during the incubation of cloud 2

	Acetate	Formate	Succinate	Oxalate	Malonate	Formaldehyde
Incubation regime	Rate of transformation ($\times 10^{-11}$ M·s^{-1})					
Reference*	0	0	0	0	0	0
Light	0	0	0	−4.0 (0 h to 60 h)	**0.3 (0 h to 60 h)**	**0.2**
Microorganisms	−15.5	−17.5 (48 h to end)	−4.5	0	−4.2 (36 h to end)	−0.3
Microorganisms + Light	−15.6	−16.1 (48 h to end)	−3.5	−2.7 (0 h to 60 h)	−4.3 (36 h to end)	0

A negative value indicates the disappearance of the organic compounds from the medium. Values in bold represent production of the compounds in question. In the case where a noncontinuous transformation occurred, the time period used for the linear regression is indicated in parenthesis.

* Reference: sterilized sample (filtration 0.22 μm) incubated in darkness.

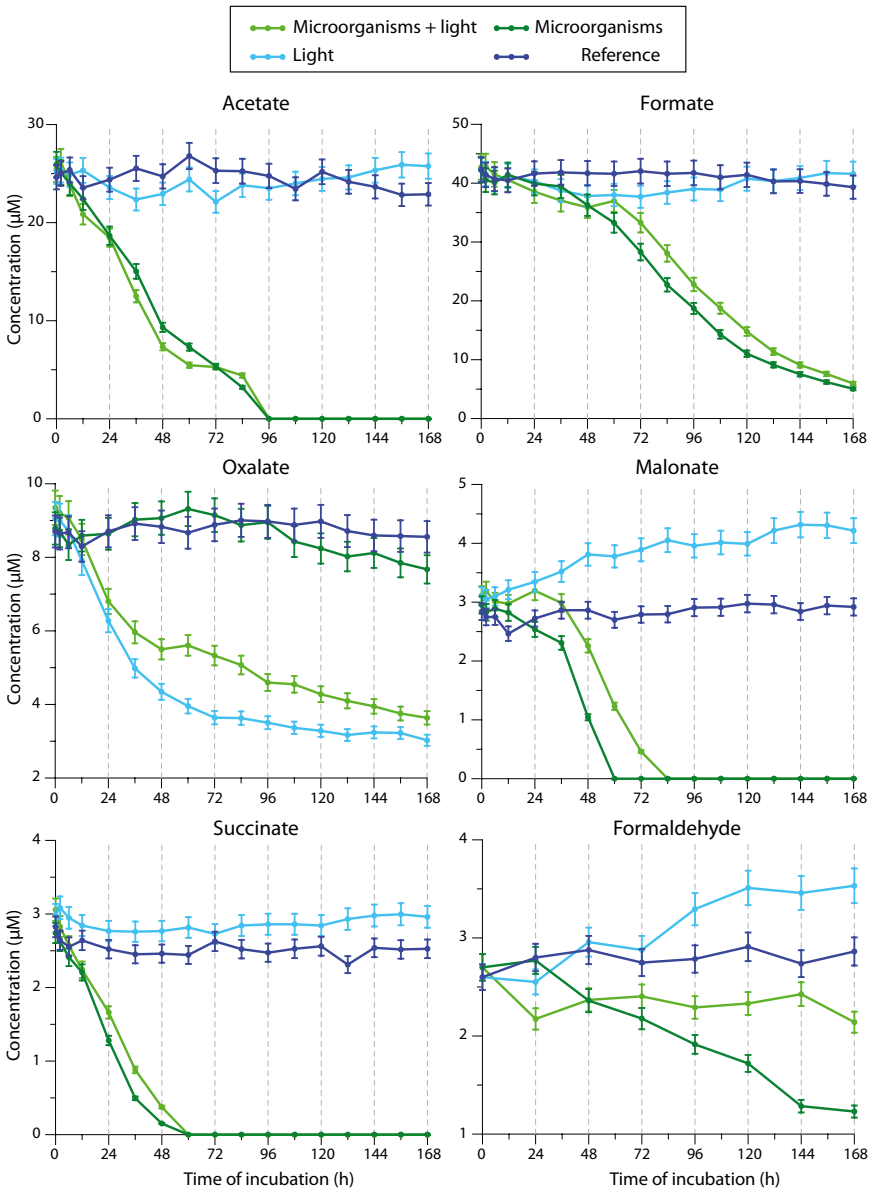

Fig. 3. Temporal evolution of carboxylic acids and formaldehyde concentrations during the incubation of cloud 2. The cloud 2 water sample was incubated at 17 °C under four incubation regimes for 7 d: unfiltered and in the presence or absence of UV radiation (Microorganisms + Light and Microorganisms, respectively), filtered and in the presence or absence of UV radiation (Light and Reference, respectively). Error bars represent the SEs of the chemical analysis (5%).

the chemical composition of clouds, which can favor the survival of some species over others (11). During their transport over long distances, these microorganisms have been subjected to numerous stresses, including evaporation-condensation cycles; nevertheless, they remained active, as shown by their good energetic states (ADP/ATP ratios range to 0.3–1.4) and their efficiency at biodegradation. Microorganisms can adapt very easily to changing conditions and stresses in the atmosphere, as observed in other extreme environments (36).

Our experiments were conducted in innovative microcosms that were designed to mimic more realistic environmental cloud conditions. Using unfiltered samples in a homemade photobioreactor, we investigated the activities of microorganisms that were exposed to UV light and H_2O_2, which is a major source of $^{\bullet}OH$ radicals in cloud waters. The experiments that combined both the photo- and biodegradation processes were compared with experiments with biodegradation (absence of UV light) or photodegradation alone (filtered sample).

First, our results indicate that the microorganisms that were present in the cloud samples and exposed to UV light remained metabolically active in the presence of $^{\bullet}OH$ radicals that were photoproduced from H_2O_2 because of the oxidative stress metabolism of the cells. This phenomenon is clearly demonstrated by the similar ADP/ATP ratios that were measured when the microorganisms were exposed or not exposed to UV light, indicating that the microbial energetic state was unchanged. This result was also clearly demonstrated by the degradation rates under combined conditions (Microorganisms + Light), where photodegradation and biodegradation are additive processes. There was no inhibition of microbial activities toward the organic, biodegradable compounds (acetate, formate, succinate, malonate, and formaldehyde) that were tested in the presence of reactive oxygen species. This information is particularly important when considering the potential role of microorganisms in cloud chemistry and the resulting carbon balance. Previously, studies were conducted in the absence of such reactive oxygen species, and the resulting biodegradation rates were subject to debate.

The results obtained here reinforce the hypothesis that the actual activity of microorganisms in clouds is an alternative route in photochemistry. These two transformation processes could coexist in cloud droplets and be modulated by the bio-physicochemical conditions that are encountered in natural warm clouds.

Second, and most importantly, we have demonstrated that H_2O_2, a precursor to oxidant species in clouds, is biodegraded by the endogenous microflora through the actions of catalases. To our knowledge, this report of such an effect in the atmospheric environment is unique. Moreover, the biodegradation process is significant compared with the photochemical process. This finding has major consequences for atmospheric chemistry because it shows that microorganisms may have an impact on the oxidant capacity of clouds. This concept is clearly unique and should be considered in much greater detail.

However, we are aware that our microcosms are still unlike real cloud systems, which are polydisperse, with highly variable spatial and temporal parameters. However, this work suggests that microorganisms could play a double role in atmospheric chemistry and, more specifically, on the carbon budget of the atmosphere. First, they could directly metabolize organic carbon species. Second, they could destroy a portion of the source of radicals because of their oxidative metabolism, and as a result, these molecules, such as H_2O_2, would no longer be available for photochemical or other chemical reactions.

Materials and Methods

Three cloud water samples from three different origins were collected at the puy de Dôme station in 2010 and were analyzed chemically and biologically. The samples were incubated in photo-bioreactors at 17 °C under four incubation regimes for 7 d: unfiltered and in the presence or absence of UV radiation (Microorganisms + Light and Microorganisms, respectively), and filtered and in the presence or absence of UV radiation (Light and Reference, respectively). We recorded the concentrations of formate, acetate, oxalate, succinate, malonate, formaldehyde, H_2O_2, and the ADP/ATP ratio during the incubation period. Additional details about the methodology are provided in *SI Text*.

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Supporting Information

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SI Text

Cloud Water Collection. Cloud water was sampled from the top of the puy de Dôme mountain (1,465 m above sea level, 45°46′ North, 2° 57′ East, France), as described on the Web site: http://wwwobs.univ-bpclermont.fr/SO/beam. The study location is frequently covered by clouds and is disconnected from local pollution. Two cloud water samplers, developed by Kruisz et al. (1), were used with an estimated cutoff diameter of 7 μm, for an approximately debit rate of 80 $m^3 \cdot h^{-1}$ (2, 3). The collection mechanisms on the cloud water samplers were sterilized using an autoclave (20 min at 121 °C) before sampling, and the body parts were sterilized on site by washing them with ethanol [70% (vol/vol)] and extensively rinsing them with sterile, ultrapure water. After 30 min, to assess the microbial sterility and chemical purity of the freshly cleaned body parts, 50 mL of sterilized, ultrapure water were poured into the sampler and recovered in the collection parts ("blank" samples). For the three cloud-sampling events presented in this article, no microbial or chemical contamination was observed in the "blank" samples.

During sampling, the cloud water was recovered from the collector under sterile conditions when the volume in each collector reached 100 mL. A minimum volume of 600 mL of cloud water was necessary for the biological and chemical analyses listed in Table 1. After collection, samples were immediately frozen or kept at 4 °C. Laboratory experiments were performed less than 2 h after the end of sampling.

Cloud Water Incubation. Half of the sample was sterilized by filtration (filter porosity 0.22 μm, nylon filter) to eliminate microorganisms. The filtered and unfiltered samples were incubated for 7 d in the dark or under UV light. The experiments were carried out at a constant temperature of 17 °C, a value slightly different from the temperature during the cloud-sampling events (10 °C, 13.5 °C, and 10 °C, for clouds 1, 2, and 3, respectively). The incubator chamber was equipped with a stirring plate (aerobic condition, 110 rpm) under a fluorescent tube that emitted radiation with wavelengths between 340 and 420 nm (λ_{max} = 365 nm and total light energy: 33 $J \cdot s^{-1} \cdot m^{-2}$). For incubation in darkness, two brown 250-mL Erlenmeyer flasks were used. For incubation under UV light emission, two photo-bioreactors were used (Fig. S1). These photo-bioreactors consisted of a cylindrical Pyrex crystallizer (300 mL, 95-mm diameter) covered with a Pyrex glass filter (3.3-mm thick and 80 mm Ø) and mounted on a nylon lid that was equipped with eight vents (Teflon tubes of 8 mm Ø, plugged with sterile cotton wool to avoid microbial contamination) to prevent water condensation on the filter and to maintain continuous oxygenation in the tested medium. Preliminary tests in artificial cloud-water solutions have shown that there was no effect of the incubation flask type on the transformation rates. Biological and chemical analyses were conducted after the cloud water collection and at various times during the incubation, as indicated in Table 1.

Chemical Analyses. ***Conductivity, pH, total and dissolved organic carbon.*** Conductivity and pH were measured on-site with a portable multiparameter pH-meter that was equipped with a temperature sensor. The total organic carbon (TOC) and dissolved organic carbon (DOC) were measured with a TOC analyzer (TOC 5050A, Shimatzu). Five milliliters of filtered (filter porosity 0.22 μm) and nonfiltered samples were used for DOC and TOC measurements, respectively, as described in Parazols et al. (4).

Fe(II), Fe(III). The iron (Fe) concentrations [Fe(II) and Fe(III)] were measured with a spectrophotometric assay with a colorimetric complexant (ferrozine) (5) that was used for cloud water analyses by Parazols et al. (4). To determine the concentrations of Fe(II) and Fe(Total), 2 × 1-mL samples were necessary. The uncertainty in the measurements was less than 10%, and the detection limit (DL) was 0.1 μM (calculated as three times the SD of the field blanks).

Ionic species. The ion concentrations in the cloud water samples were measured using ionic chromatography (3, 6) units: Dionex DX320 for anions (column AS11, eluent KOH) and Dionex ICS1500 for cations (column CS16, eluent hydroxymethanesulfonate acid). Samples were thawed 15 min before their dilution by a factor of 10 in ultrapure water and transferred into vials (5 mL) previously rinsed with ultrapure water. No chemical transformations were observed in our samples after one freeze/thaw cycle. The accuracy of the ion chromatographic analyses was 5% (3).

Formaldehyde. The formaldehyde concentration was measured using a miniaturized fluorimetric assay that was adapted from Li et al. (7). The reaction medium included 60 μL of ammonium acetate solution (4 M), 60 μL of acetoacetanilide [0.2 M in ethanol solution 50% (vol/vol)], 60 μL of ethanol (96°) and 120 μL of the cloud water sample. The solutions were mixed and incubated on a plate with 96 black, flat-bottomed wells at room temperature for 25 min before reading (λ_{ex} = 375 nm and λ_{em} = 490 nm). The uncertainty in the measurements was less than 5%, and the DL was 0.1 μM (calculated as three times the SD of the field blanks).

H_2O_2. The hydrogen peroxide concentration was measured using an accurate enzymatic fluorimetric assay with a 4-Hydroxyphenylacetic acid that produced a fluorescent dimeric compound with hydrogen peroxide [microassay adapted from Lazrus et al. (8)]. Next, 1.5 mL of a solution of 4-Hydroxyphenylacetic acid (1.5 mM) in a phosphate buffering solution (0.1 M, pH 7.4) was mixed with 10 μL of a horse radish peroxydase solution (380 units/mL^{-1}) to constitute the reagent solution; this solution was kept at 5 °C for less than 12 h. After sampling (less than 5 min), 10 and 50 μL of the cloud water sample were mixed on-site with 200 μL of the reagent solution in duplicates and incubated at room temperature (>17 °C) for 5 min before freezing at −25 °C. A new calibration was systematically performed before each analysis session, with a normalized H_2O_2 solution with a different concentration (0–200 μM) using the same reagent lot. Before the analysis, the mixed samples (cloud water + reagent) were thawed at ambient temperature for 10 min, and 200 μL were analyzed. Previous tests indicated that the fluorescent dimeric compound remained stable after this freeze/thaw process. Fluorescence readings (λ_{ex} = 320 nm and λ_{em} = 390 nm) were made in a 96-well format. The uncertainty of the measurements was less than 5%, and the detection limit was 0.07 μM.

To determine the concentration of organic peroxides, samples were treated with catalase. After 30 s, 200 μL of the reagent solution was added, and the previous protocol was applied. For all cloud waters, the organic peroxide concentrations were lower than the limit of detection (0.07 μM).

Biological Analyses. A direct enumeration of cells (duplicates, 10 mL) was performed with epifluorescence microscopy, as described by Amato et al. (9). The main difference in methods was in the use of a phosphate buffering solution (pH = 7) to improve the fluorescence efficiency.

The ATP and ADP concentrations were measured in cloud water samples (0.2 mL) using the ATP Biomass Kit HS (Biothema) and a Biolumineter (Lumac Biocounter M2500). The analytical protocol was described in ref. 10.

The cloud water samples (0.2 mL) were strongly mixed in situ in a sterile microtube with an equal volume of extractant B/S, from the ATP Biomass Kit HS (Biothema). This mixture was used for ATP determination and was stored frozen before the analysis. The ATP concentrations were determined by bioluminescence (11, 12) using a Biolumineter (Lumac Biocounter M2500). The ADP concentration was determined after the direct transformation of ADP to ATP in the luminometer tube, in the presence of pyruvate kinase and phosphoenolpyruvate; this analytical protocol is described in ref. 10. A small volume of the mixture (sample + extractant B/S: 60 μL) was used to determine the ATP or ADP concentration.

Calculations of the Transformation Rates. To calculate the initial transformation rates, the temporal evolution of the concentration of each compound was plotted. Then, the pseudofirst order decay "k" (s^{-1}) was determined (only for $r^2 > 0.8$) using the following linear regression:

$$\ln([C]/[C]_0) = f(t) = -k \times t.$$

The transformation rate of the compound C (v_c) was determined as follows:

$$v_c = k \times [C]_t \quad [M^{-1}\ s^{-1}],$$

where $[C]_t$ is the concentration of the chemical compound C ($mol \cdot L^{-1}$) at time t (s), and k is the pseudofirst order decay (s^{-1}).

H_2O_2 Degradation: Relative Contribution of Biotic and Abiotic Processes. To quantify the relative impact of biotic activity compared with abiotic H_2O_2 transformations, we corrected some of our data. We used the initial degradation rate of H_2O_2 in the absence of microorganisms and light (reference sample) to quantify the impact of the radical reactions that were nonphotochemically induced. For photodegradation and biodegradation, we have subtracted this value from the initial rates that were measured in the presence of light, or microorganisms, respectively (Table S2). From these corrected values, it was possible to calculate the relative contribution of each type of reaction (Fig. S3).

Bio- and Phototransformation of Carboxylic Acids and Formaldehyde in Cloud Samples. The concentrations of formate, acetate, succinate, oxalate, malonate, and formaldehyde were recorded at 17 °C under four incubation conditions ("Microorganisms + Light," "Light," "Microorganisms," and "Reference") for the three cloud water samples (Fig. 3, and Figs. S4 and S5). The transformation rates that were measured in these experiments are listed in Table S3.

Under dark conditions with the endogenous microbial population (Microorganisms case), the acetate and formate biodegradation rates were much higher than those of succinate, malonate, and formaldehyde for the three cloud events. For formate, a lag time was observed in clouds 1 and 2, as already noted for cloud 2. For malonate, two kinetic steps took place in clouds 2 and 3: until 36 h, the rates were slow but then increased dramatically. Oxalate was not biodegraded in any of the samples; this lack of degradation was also observed with selected bacterial strains that were isolated from cloud water (13).

Under UV light conditions (Light) with filtered cloud water, only the succinate concentrations did not evolve. Formaldehyde was continuously photoproduced, a result of the production of $^{\bullet}OH$ from H_2O_2 photolysis. Malonate was photoproduced up to a stationary concentration (60 h) (clouds 2 and 3). Oxalate was photodegraded up to a stationary state (60 h) that corresponded to the consumption-time of H_2O_2 in these samples (clouds 2 and 3). Formate was slowly degraded in cloud 3, up to 48 h, and was not degraded in cloud 2. The behavior of cloud 1 was somewhat different; a slow photoproduction of acetate and formate was observed, but oxalate was degraded continuously until the end of the experiment and was correlated with the presence of H_2O_2. The variations in the rates of organic compound phototransformation were related to the initial H_2O_2 concentrations in the three cloud waters (Tables 1 and 2). Consequently, the end of these transformations was linked to the total consumption of H_2O_2. In summary, from the observations that were made during incubations under UV light, the temporal evolutions of organic compound concentrations were highly variable, resulting from both the photoproduction and photodegradation processes. For cloud 1, the photodegradation rate was very low because the H_2O_2 concentration was 10–20 times lower than that in clouds 2 and 3, respectively.

In the presence of UV light and microorganisms (Microorganisms + Light case), we observed the sum of photochemical and microbial activities without synergy or inhibition. In clouds 2 and 3, the degradation rates of acetate, formate, and succinate, were similar with and without UV light because these compounds were not involved in any photochemical processes. For oxalate, the degradation rates were the same for light alone and light plus microorganisms; this observation is in agreement with the nonbiodegradability of this compound (clouds 2 and 3). Finally, for malonate, production and destruction are competitive (clouds 2 and 3). During the first part of the kinetic (up to 48 h), photoproduction dominated. Then, after 36 h, biodegradation began and became dominant. A similar behavior was noted for the degradation of acetate in cloud 1. The first photoproduction occurred before 72 h, and biodegradation then took over the relay. Formaldehyde was photoproduced and biodegraded. The apparent nontransformation of formaldehyde in the cloud waters in the presence of UV light and microorganisms (especially for clouds 2 and 3) revealed the competition between these two processes.

For all experiments, the organic compound biotransformation rates were similar between incubation under UV light or in darkness, and we did not observe an inhibition effect of reactive oxygen species over the microbial carbon metabolism.

Estimation of the Global Consequences of Microorganisms in Carbon Flux in the Atmosphere. From the number of cells (Table 1) and the degradation rates that were measured in the unfiltered microcosms that were incubated in the dark (i.e., the biodegradation rates, converted into $gC\ h^{-1}\ cell^{-1}$) (Table 3 and Table S4), we inferred the consumption of dissolved organic carbon species by microorganisms in clouds at the global scale (Table S4). Assuming a total mass of clouds of 1.94×10^{17} g on earth (14), microorganisms would constitute between 13 and 60 million tons of DOC, originating from formate, acetate, oxalate, malonate, succinate, and formaldehyde each year, on a global scale (results are based on clouds 1 and 2, respectively, in which the extreme lower and upper levels of microbial activity were measured). This is a conservative estimate of the total dissolved organic carbon biodegradation in clouds because other carbon compounds not measured here were most likely also consumed by cells. If we assume a bacterial growth efficiency on those compounds of 0 (i.e., these carbon sources are not used by microorganisms for producing biomass but are completely respired into CO_2), which is close to reality at low carbon concentrations (15), then the microbial respiration would lead to a global release of 51–215 million tons of CO_2 per year.

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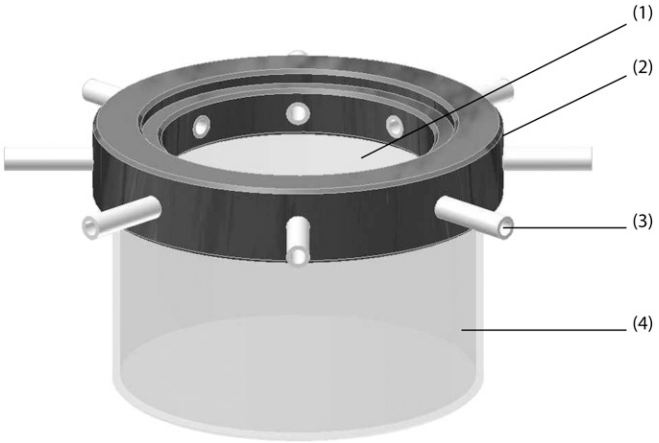

Fig. S1. Photo-bioreactor used for cloud water incubation under UV light . 1, Pyrex filter; 2, nylon lid; 3, Teflon tube of 8 mm Ø plugged with sterile cotton (eight vents); 4, cylindrical Pyrex crystallizer.

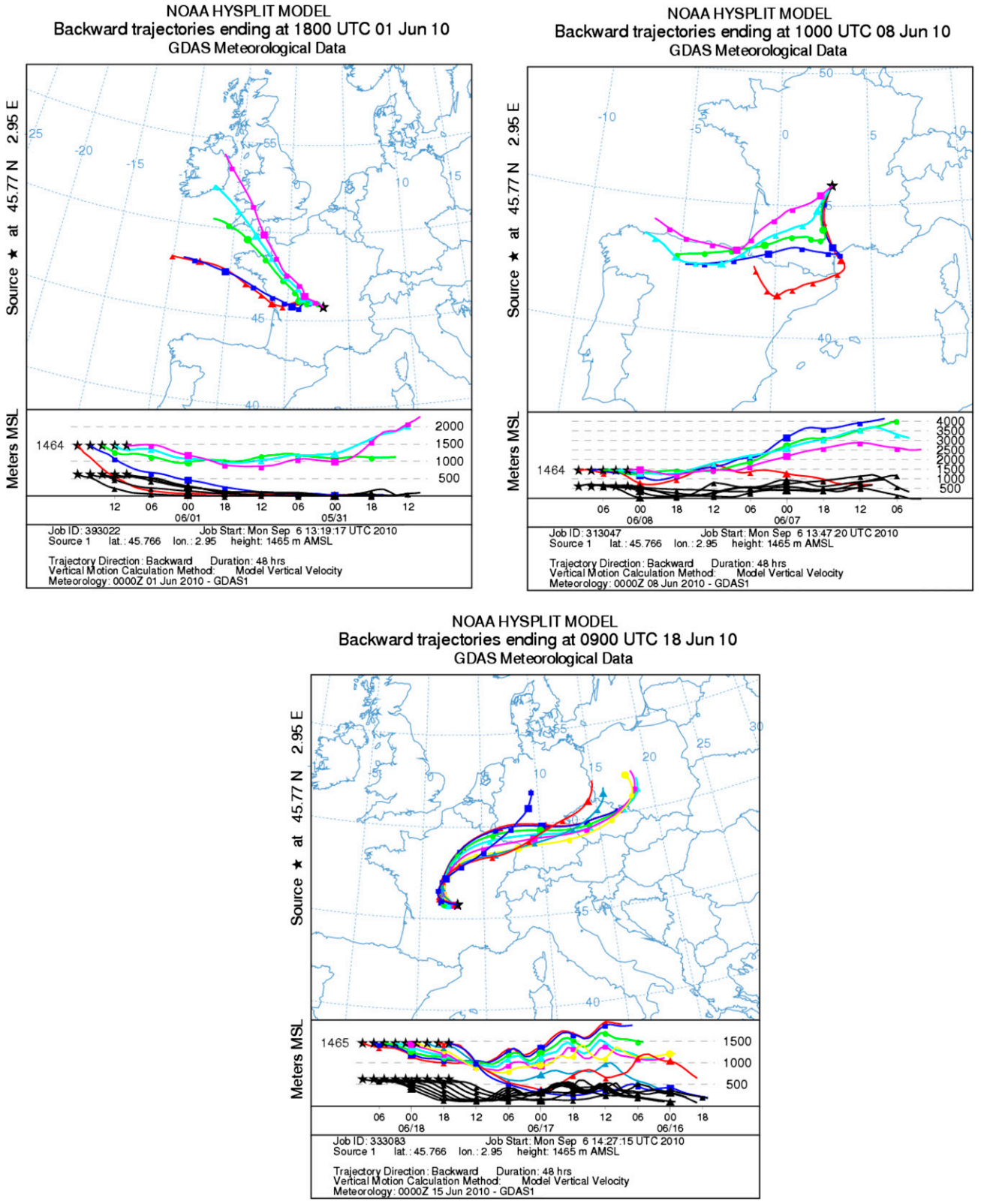

Fig. S2. The 48-h backward trajectories from the National Oceanic and Atmospheric Administration Hysplit model of clouds 1, 2, and 3.

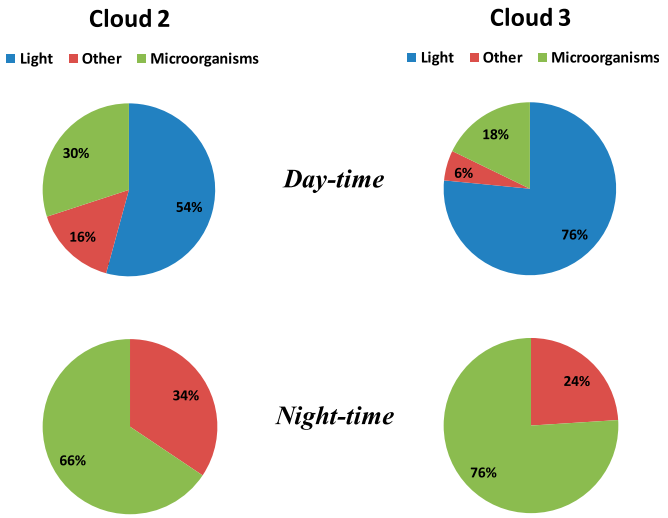

Fig. S3. Relative contribution of biotic and abiotic processes to H_2O_2 degradation during the incubation of clouds 2 and 3. "Light" (in blue) corresponds to pure photochemical processes; "Other" (in red) corresponds to nonphotochemically induced radical processes; and "Microorganisms" (in green) corresponds to a pure biodegradation processes. Percentages were calculated from the initial rates of degradation that were reported in Table S2.

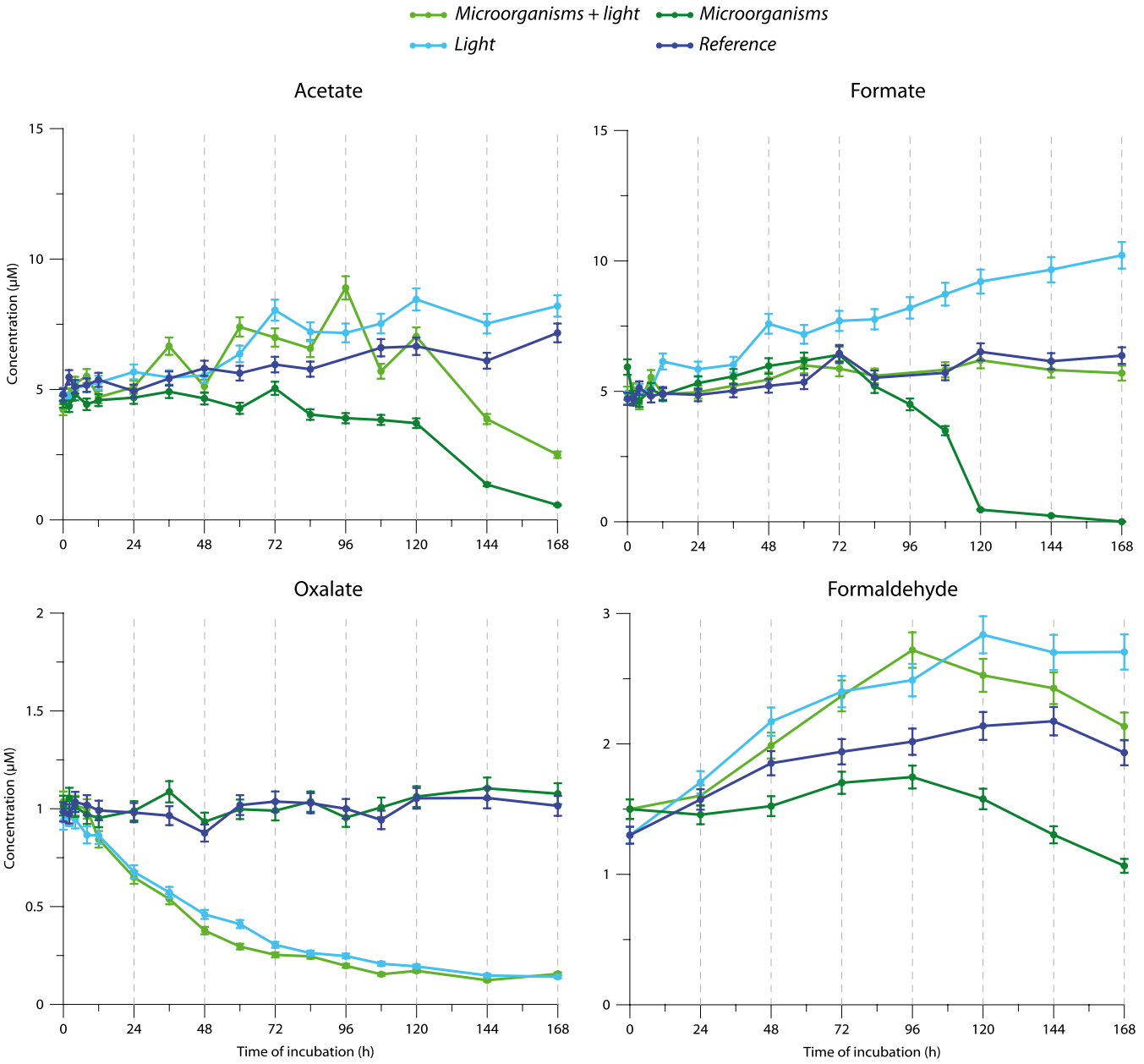

Fig. S4. Temporal evolution of carboxylic acids and formaldehyde concentrations during the incubation of cloud 1. The cloud 1 water sample was incubated at 17 °C under four incubation regimes for 7 d: unfiltered and in the presence or absence of UV radiation (Microorganisms + Light and Microorganisms, respectively), filtered and in the presence or absence of UV radiation (Light and Reference, respectively). Error bars represent the SEs of the chemical analysis (5%).

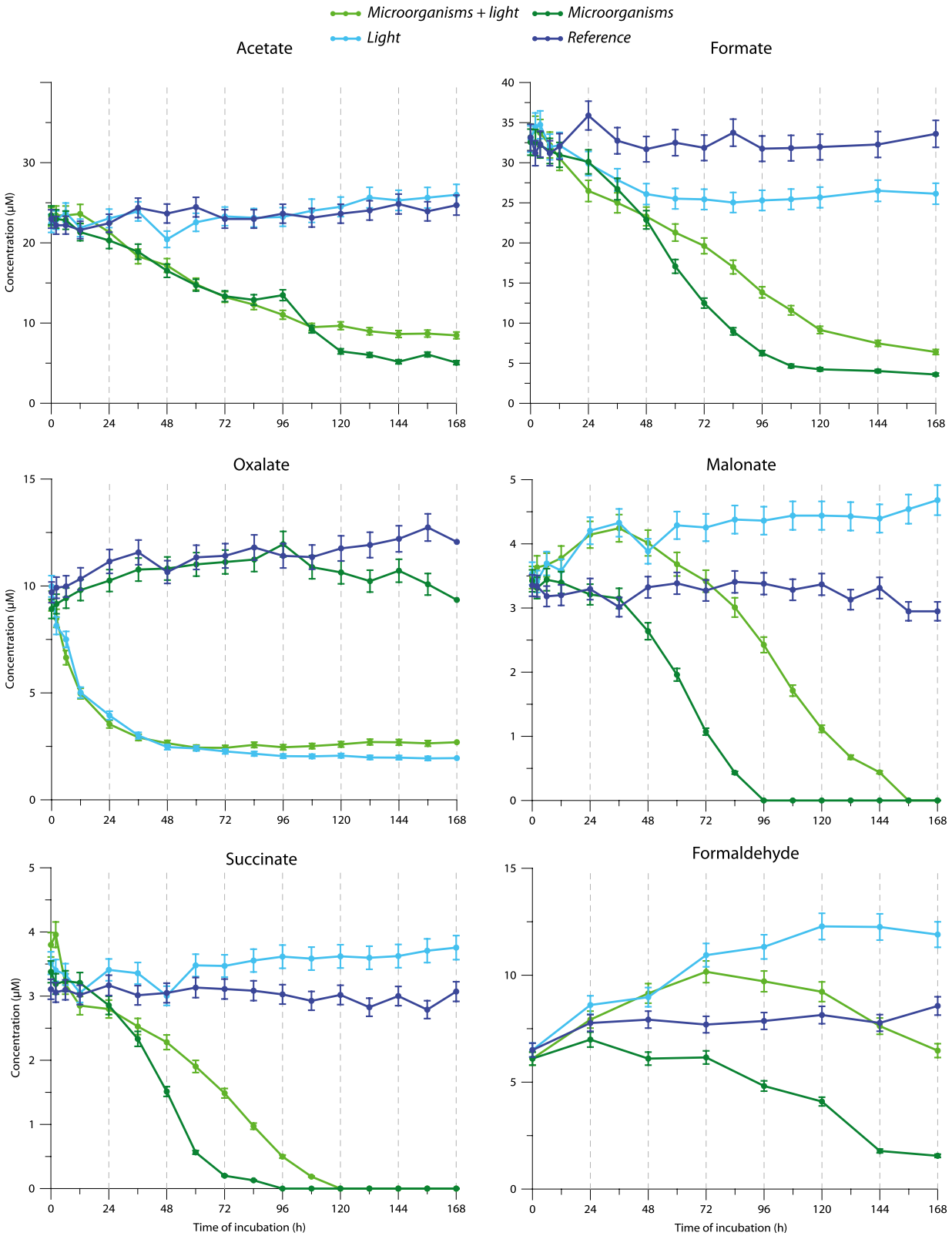

Fig. S5. Temporal evolution of carboxylic acids and formaldehyde concentrations during the incubation of cloud 3. The cloud 3 water sample was incubated at 17 °C under four incubation regimes for 7 d: unfiltered and in the presence or absence of UV radiation (Microorganisms + Light and Microorganisms, respectively), filtered and in the presence or absence of UV radiation (Light and Reference, respectively). Error bars represent the SEs of the chemical analysis (5%).

Table S1. Bio-physico-chemical measurements in cloud water samples

Measurements	Immediately after sampling	During incubation time			
		Start	Every 12 h	Every 24 h	End
PH	■	■		■	■
Conductivity	■	■			■
TOC	■	■			■
Ionic chromatography	■	■	■	■	■
Fe(II)/Fe(III) assay	■	■			■
H_2O_2 concentration	■	■	■	■	■
Formaldehyde assay	■	■	■	■	■
ATP/ADP*	■	■	■	■	■
Total cells counts	■	■			■
Cultivable cells counts		■			■

After 2 and 6 h of incubation, ionic chromatography analyses and H_2O_2 assays were performed for each sample.
*Only performed at the beginning and end of the incubation for the filtered (sterilized) samples to assess the sterility.

Table S2. Calculated values of the initial transformation rates of H_2O_2, linked to biotic and abiotic processes occurring during the incubation of clouds 1, 2, and 3

Process	Cloud 1	Cloud 2	Cloud 3
	Initial rate of H_2O_2 transformation ($\times 10^{-11}$ M·s^{-1})		
Abiotic			
Light	−0.9	−33.8	−97.9
Other	0	−9.8	−7.2
Biotic			
Microorganisms	0	−18.7	−22.8

"Light" corresponds to pure photochemical processes, "other" corresponds to nonphotochemically induced radical processes, and "microorganisms" corresponds to pure biodegradation processes. Negative values indicate the disappearance of H_2O_2 from the medium.

Table S3. Initial transformation rates of carboxylic acids and formaldehyde in the presence and absence of UV light and/or microorganisms during the incubation of cloud 2

Event no.	Parameters	Acetate	Formate	Succinate	Oxalate	Malonate	Formaldehyde
		Rate of transformation ($\times 10^{-11}$ M·s^{-1})					
Cloud 1	Light	**0.5**	**0.7**	—	−0.4	—	0.2
	Microorganisms	−2.3 (72 h to end)	−13.9 (72 h to end)	—	0	—	−0.3 (60 h to end)
	Microorganisms + Light	0.7 (0 h to 84 h)	0	—	−0.4	—	0.3 (0 h to 96 h)
		−0.9 (84 h to end)					−0.2 (96 h to end)
Cloud 2	Light	0	0	0	−4.0 (0 h to 60 h)	**0.3** (0 h to 60 h)	0.2
	Microorganisms	−15.5	−17.5 (48 h to end)	−4.5	0	−4.2 (36 h to end)	−0.3
	Microorganisms + Light	−15.6	−16.1 (48 h to end)	−3.5	−2.7 (0 h to 60 h)	−4.3 (36 h to end)	0
Cloud 3	Light	0	−2.6 (0 h to 48 h)	0	−8.5 (0 h to 48 h)	**0.5** (0 h to 48 h)	0.9
	Microorganisms	−5.8	−12.5	−3.4	0	−3.5 (36 h to end)	−1.0
	Microorganisms + Light	−4.4	−8.5	−2	−8.0 (0-48 h)	0.6 (0 h to 36 h)	0
						−2.4 (36 h to end)	

A negative value indicates the disappearance of the organic compounds from the medium. Values in bold represent production of the study compounds. In the case where a noncontinuous transformation occurred, the time period used for the linear regression is indicated in parenthesis. A dash indicates no transformations observed for the "Reference" sample (i.e., sterilized sample incubated in darkness).

Table S4. Estimates of the amount of CO_2 released by microbial respiration in clouds

Observed and inferred	Cloud 1	Cloud 2	Cloud 3
Observed in natural clouds			
Cell concentration (mL^{-1})	$3.0\ 10^{4}$	$8.0\ 10^{4}$	$9.0\ 10^{4}$
Carbon biodegradation rate (gC h^{-1} $cell^{-1}$)	$2.71\ 10^{-13}$	$4.29\ 10^{-13}$	$3.28\ 10^{-13}$
Inferred at global scale*			
Total number of cells in clouds	$5.82\ 10^{21}$	$1.55\ 10^{22}$	$1.75\ 10^{22}$
Carbon biodegradation rate (gC h^{-1})	$1.58\ 10^{9}$	$6.65\ 10^{9}$	$5.73\ 10^{9}$
Carbon biodegradation rate (tons of C yr^{-1})	$1.38\ 10^{7}$	$5.83\ 10^{7}$	$5.03\ 10^{7}$
Amount of CO_2 released by microbial respiration (tons yr^{-1})	$5.06\ 10^{7}$	$2.14\ 10^{8}$	$1.84\ 10^{7}$

The cell concentrations are listed in Table 1, and the degradation rates from Table 3 were converted into gC h^{-1} $cell^{-1}$. The total mass of clouds follows the conclusions of Pruppacher and Jaenicke (14).
*Total mass of clouds = 1.94×10^{17} g (14).